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PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY

FORTY-SEVENTH ANNUAL MEETING

Detroit, Mich., April 10, 11, 12, 13, 1935

The circulatory conditions required for urine formation. E. F. ADOLPH.
Department of Physiology, School of Medicine and Dentistry, The University of Rochester.

One ureter was cannulated, and the flow of blood was watched in the glomeruli of the kidney of the same side, in frogs whose cerebra and mid-brains were destroyed. Heart rates were also counted, and observations, including rates of urine flow, were ordinarily made at intervals of 1 minute. All the known means of decreasing the rate of urine formation were tested: low oxygen tensions, high carbon dioxide tensions, four anesthetics, concentrated salt solutions on the skin, drying the skin, and subcutaneous pituitrin.

All these agents either stopped the flow of blood in some or all glomeruli, or decreased the rate of heart beat, or both. The flow in the renal-portal system was unchanged. When the blood pressure was measured in the aorta it was found to change in parallel with the heart rate under several of these experimental conditions. When the blood pressure in glomeruli was measured, by suddenly imposing sufficient pressure in the ureter to stop the flow of blood in the glomerular capillaries, this also changed in phase with the heart rate, but it often decreased more than the aortic pressure decreased. Cutting nerves to the heart or to the kidney made no difference in the responses.

The time relations of the various changes were compared. The heart rates and glomerular blood flows were usually modified at the same time that the rate of urine excretion changed, and within 3 minutes of the administration of the anuric agent. Sometimes the blood flow was modified in advance; the urine rates never decreased or increased *before* the heart rate did or the glomerular blood flow did, in more than 50 instances that were accurately observed.

In general, all the agents that produced anuria characteristically either generally or locally impeded the flow of blood and lowered its pressure in the glomeruli. At the same time, particular instances were found in which oliguria or anuria occurred without visible diminution in general circulation or in glomerular blood flow. The circulatory changes either were simultaneous with, or preceded, by intervals of 1 to 20 minutes, the changes in rate of urine formation.

Some effects of pitressin on tissue water and insensible water loss. ERRETT C. ALBRITTON, RIETTA G. ALBRITTON (by invitation) and WADE H. MARSHALL (by invitation). Department of Physiology, The George Washington University Medical School.

Unanesthetized fasting rats were given subcutaneous injections of 0.006 pressor unit of pitressin solution per 100 grams of body weight and were sacrificed at different intervals thereafter. Samples of skin, muscle, liver, spleen and blood were taken. Water content of each was determined by weighing before and after drying for 36 hours at approximately 100°C. in a stream of dry carbon dioxide.

Measurements from 50 experimental animals showed a definite increase in skin water content as compared with controls. Observations were made as late as 10 hours after the administration of the pitressin and the difference was still observed. Blood and liver water showed a suggestive decrease, muscle and spleen water an increase. Changes were apparently without relation to urine excretion, hemorrhage, saline injected intraperitoneally, or presence or absence of the suprarenals.

Insensible water loss, as measured by means of wet and dry thermocouple thermometers in a stream of air passing over the animal is under investigation.

The inhibition in vivo of oestrous motility of the uterus in unanesthetized rabbits by crystalline progestin. WILLARD M. ALLEN and S. R. M. REYNOLDS. Department of Obstetrics and Gynecology, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y., and the Department of Physiology and Pharmacology, Long Island College of Medicine, Brooklyn, N. Y.

In a previous paper (Reynolds and Allen, This Journal **102**: 39, 1932) we have shown that crude progestin-containing extracts of the corpus luteum inhibit spontaneous oestrous motility as recorded by an intrauterine balloon in the unanesthetized rabbit. The present experiments were carried out to compare the effect of crystalline progestin (B and C, $C_{21}H_{30}O_2$) with that of the impure extracts. Castrated rabbits were used in which oestrous motility had been induced by the injection of 100 rat units of Theelin, half intravenously and half subcutaneously, in three divided doses on the day preceding the test. It was found that a given impure fraction known to contain progestin caused inhibition of oestrous motility in vivo in 4 hours when 0.3 rabbit unit was given, 2 hours with 0.6 rabbit unit, and 1 hour with 1.2 rabbit units, under the conditions of the experiment. Similar doses of crystalline progestin B (short prisms melting at 123.5-125°, C 80.16 per cent, H 9.68 per cent) and progestin C (long needles mp. 120.5-121°, C 79.58 per cent, H 9.58 per cent) gave inhibition times which were almost identical with those for the crude extract. (The two forms of progestin probably represent polymorphous forms of the same substance, Wintersteiner and Allen, J. Biol. Chem. **107**: 321, 1934.)

These results show that crystalline progestin inhibits oestrous motility in vivo in the unanesthetized rabbit in the same manner as crude progestin and with similar doses. They also demonstrate that progestin has at least two functions—one, that of inducing progestational proliferation of the endometrium, and two, that of causing uterine quiescence.

Shivering and the cerebral cortex. CHARLES D. ARING (introduced by J. F. Fulton). Laboratory of Physiology, Yale University School of Medicine, New Haven.

Monkeys and baboons with various ablations of limited areas of the cerebral cortex, or with lesions of the cerebellum, were placed suddenly in a reduced environmental temperature; the shivering response and rectal temperatures were compared with those of normal animals in the same circumstances and with the same animals before operation. Significant deviation of these responses from the normal were found in all animals with partial or complete unilateral or bilateral ablation of the motor area (area 4). In this group there quickly occurred vigorous, symmetrical shivering in response to cold, frequently before a drop in the rectal temperature.

Primates with ablations of the following areas (using Brodmann's numerical designations as modified by the Vogts): unilateral and bilateral premotor (area 6), and frontal association (areas 9-12), bilateral post-central gyrus (sensory areas 3, 1, 2), bilateral parietal association (area 7), and unilateral temporal lobe (areas 20, 21, 22) showed temperature and shivering responses to cold, which were entirely comparable to those of the normal animal. The animals in this group showed the usual drop in rectal temperature after prolonged exposure to reduced temperatures. They then shivered mildly and intermittently. Animals with unilateral section of the cerebellar peduncles exhibited shivering ipsilaterally which appeared irregular in pattern, and possibly mildly uncoördinated as compared with that of the opposite extremities. In one animal a chordotomy severing the rubrospinal tract, prevented shivering in the region innervated from behind the lesion. It is concluded that normal inhibition of the shivering response is mediated through the motor projection fibres from area 4.

Anti-gonadotropic substances. CARL BACHMAN (by invitation), HANS SELYE, D. L. THOMSON and J. B. COLLIP. Department of Biochemistry and Pathological Chemistry, McGill University.

The serum of rats, rabbits and sheep treated with gonadotropic hormones from various sources contains substances capable of influencing the responses of male and female test animals to these hormones. The specificity, nature of the modifying action, and the methods of assaying such anti-gonadotropic sera will be briefly discussed.

The effect of glucose and fructose separately and in combination on the respiratory exchange and some urinary constituents, at rest. GEORGE BACHMANN and JOHN HALDI. Laboratory of Physiology, Emory University School of Medicine.

Although a number of workers have investigated the effect of glucose, fructose and these sugars in combination on the respiratory exchange it was deemed advisable to repeat this work on our two subjects to obtain preliminary data requisite for certain other investigations which we propose to undertake.

The R.Q.s of the post-absorptive state were determined for three successive 15 minute periods. The sugars were then ingested and the respiratory exchange followed at 15 minute intervals for $1\frac{3}{4}$ hours, with the subject

in the recumbent posture. In general, the same results were obtained with the two subjects.

Since the ingested sugars were dissolved in 500 cc. water at 37°C., experiments were carried out to determine the influence of the ingestion of this volume of water. In these experiments the R.Q. rose, during the second 15 minute period following ingestion, from an average basal level of 0.76 to an average of 0.81 with one subject, and from 0.80 to 0.83 with the other subject. It then dropped gradually to 0.77 and 0.81 respectively, $1\frac{1}{4}$ hours after ingestion.

The ingestion of glucose was followed by a gradual rise of the R.Q. from an average of 0.79 to 0.91 where it remained until the end of the experiment. The rise after fructose alone and glucose and fructose combined was much more abrupt and reached a higher level. With one subject it rose to an average of 1.00 with fructose and 1.01 with the combined sugars.

Urine was analyzed after a basal period of 45 minutes and again $1\frac{3}{4}$ hours after ingestion of the sugars for lactic acid, titratable acidity, creatinine, creatine, total nitrogen, ammonia, total phosphorus, inorganic and organic phosphorus. Comparisons were made on the basis of hourly excretion.

No apparent change was observed in the urinary constituents following the ingestion of the sugars, except in the case of creatine. Creatine which was excreted only 3 times in 23 experiments during the three resting periods in the post-absorptive state, appeared 11 times out of 18 experiments after the ingestion of the various sugars, fructose having apparently a greater influence in this respect.

The effect of a reduction in the blood flow through skeletal muscles on the potassium content of the venous blood plasma. ANNA M. BAETJER.
Department of Physiology, School of Hygiene, Johns Hopkins University.

Previous work indicated that stimulation of the abdominal sympathetic chain in cats caused an increase in the concentration of potassium in the plasma of the femoral venous blood. These experiments were undertaken to determine whether this increase in potassium was a direct result of sympathetic stimulation or a secondary effect following a reduction in blood flow due to the accompanying vasoconstriction.

The blood supply to the resting hind limb of cats was reduced by continuous bleeding from the femoral vein and by temporary clamping of the abdominal aorta. Successive samples of venous blood were analysed for potassium. When the blood flow was reduced the potassium content of the femoral venous blood remained fairly constant or increased slightly until the rate of venous outflow was reduced to eighty per cent of the original value. Further reduction, however, led to a marked rise in potassium, a reduction of ninety per cent causing an increase of sixty per cent in the potassium concentration.

The increase in blood potassium must represent a loss of potassium from the muscles. It was not due to concentration of the blood as dilution always occurred when the flow was reduced nor did the increase in potassium bear any direct relationship to the amount of dilution. The relationship between the excess blood potassium and the reduction in flow was not altered when allowance was made for the dilution.

Several experiments were made to determine whether a loss of irrita-

bility of the resting muscle accompanied this loss of potassium. The muscles were stimulated indirectly at intervals, before, during and after prolonged clamping of the aorta such as had been found to cause a marked increase in potassium. The height of the resulting contractions was not greatly diminished by the clamping.

The increase in potassium brought about by stimulation of the sympathetic chain when plotted against the accompanying reduction in blood flow was found to be no greater than that produced by these other methods of reducing the blood supply.

Micromethod of measuring osmotic pressure. EDWARD J. BALDES. Division of Physics and Biophysical Research. The Mayo Foundation, Rochester, Minnesota. (Demonstration.)

The micromethod of measuring vapor pressure was developed in the laboratory of Prof. A. V. Hill and is a modification of the Hill thermoelectric method in which a differential thermocouple replaces the usual thermopile. As with the previous technic, measurements are made against a standard solution of NaCl expressed as grams of NaCl per 100 grams H₂O. The solutions are applied directly to the junctions of the thermocouple and held there by surface forces in form of droplets, the size of which may be as small as 0.1 c. mm. Using thermocouples as previously indicated, the sensitivity is the same as with the thermopile and the accuracy attainable with certain precautions may be more than 0.2 per cent, which corresponds to an accuracy of 0.001°C. depression of freezing point for biologic solutions.

The advantages of the modified thermo-electric method are: 1, a differential thermocouple replaces the differential thermopile; 2, the solutions do not come in contact with filter paper but are applied directly to the insulated thermojunction; 3, measurements may be made on small quantities of solution. As with the thermopile, the determinations of vapor pressure may be carried out at body temperatures and in a gas comparable to that in equilibrium with the tissue.

The effects of denervation of the genitalia on the oestral behavior of cats.¹ PHILIP BARD. Department of Physiology, Johns Hopkins University School of Medicine.

The animals selected for these experiments were normal and ovariectomized females in which, previous to any attempt to denervate, oestrus had been repeatedly induced and maintained for weeks by feeding untreated human pregnancy urine or by intramuscular injection of Progynon-B. Experience has shown that the behavior which characterizes oestrus induced by these materials is the same as that exhibited by cats in spontaneous oestrus. It may be divided into: 1, courtship activities (playful rolling, rubbing, calling, crouching with pelvis raised and treading); and 2, the after-reaction (vigorous or frantic rolling, rubbing, squirming and licking) which follows intromission or artificial mechanical stimulation of the distal vagina. Although the courtship activities occur spontaneously, they are intensified by the presence of a male. During anoestrus vaginal stimulation is never followed by the after-reaction and

¹ Aided by a grant from the Committee for Research in Problems of Sex, National Research Council.

it is only during oestrus that crouching and treading can be induced by touching the vulval region or grasping the nape of the neck.

No modification of any aspect of the behavior attending induced oestrus was observed after abdominal sympathectomy, after removal of the uterus and proximal vagina or after a combination of these procedures. When oestrus was induced after removal of the sacral cord alone or in combination with one or both of the extirpations just mentioned, the animals exhibited all the courtship activities, permitted males to mount, but showed no response to intromission or similar mechanical stimulation of vulva and distal vagina; they never showed any trace of the after-reaction. Furthermore, no reactions of any kind could be elicited in them by nociceptive stimulation of the distal vagina, vulva, anus, tail or the skin surrounding the vulvo-anal region and tail base.

When oestrus was induced after transection of the cord at a mid-thoracic level the head, neck and forelegs engaged in typical courtship activities.

The influence of posterior pituitary extracts on experimental diabetes. B. O. Barnes. Department of Physiology, University of Chicago. (Read by title.)

Since the discovery of Houssay that hypophysectomy ameliorates severe diabetes which usually follows pancreatectomy, attention has been focussed on the anterior lobe. However, it is well established that the posterior pituitary extracts will elevate blood sugar. Since acetic acid extracts of the anterior lobe yield a potent diabetogenic principle, the same procedure was applied to the posterior lobe. To 0.60 gram of Armour's posterior pituitary powder (I am indebted to Doctor Fenger of Armour & Company for this material) were added 100 cc. of 0.25 per cent acetic acid. The mixture was heated to boiling and filtered. The filtrate was neutralized with saturated sodium bicarbonate. This extract in doses of 5 to 10 cc. daily was found to aggravate the diabetic condition of pancreatectomized, hypophysectomized-pancreatectomized, or pancreatectomized dogs in which the epinephrine secretion had been suppressed. Further work will be necessary to determine whether this principle is the same as that found in anterior pituitary extracts.

Some observations on dogs following the administration of pregnant mare's serum. B. O. Barnes and J. G. Bueno (by invitation). Department of Physiology, University of Chicago. (Read by title.)

Reports have appeared which indicate that pregnant mare's serum contains a potent gonadotropic principle for immature rat ovaries. Since the effects of this principle are in some respects similar to extracts from the anterior pituitary, this work was undertaken to see if pregnant mare's serum had any thyrotropic or diabetogenic effect on dogs.

Serum was collected from pregnant mares about 90 days after breeding. It was tested on immature rats 21 days old, by injecting 1 cc. daily for 4 days. The animals were killed on the fifth day and the ovaries and uterus were over 500 per cent heavier than the controls. The vaginal orifice was open in each case.

Administration of the mare's serum to 4 dogs, trained for basal metabolism was not followed by any increase in the B. M. R. although as much as 20 cc. per day was employed. In similar doses no diabetogenic activity could be detected when the serum was administered to a hypophysecto-

mized-depancreatized dog or a depancreatized dog in which epinephrine secretion had been suppressed. All of the above animals were known to respond to anterior pituitary injections. In three female dogs, one of which was hypophysectomized, there was no external evidence of ovarian stimulation. It was previously found (Barnes and Bueno) that the rat and the dog may differ in their response to gonadotropic preparations. Further work is being done with larger doses of mare's serum to see if the dog is refractory to this gonad stimulant.

The influence of thyroglobulin on basal metabolism. B. O. BARNES and J. G. BUENO (by invitation). Department of Physiology, University of Chicago. (Read by title.)

Various workers have reported a rise in metabolism following the injection of anterior pituitary extracts. Using the dog we previously found that an elevation of 30 to 40 per cent could be maintained for several days. Presumably the effects were due to thyroglobulin secreted in excess since a thyroidectomized dog showed no response. The total iodine content of dog thyroids averages 2 mgm. under our laboratory conditions. The daily administration of thyroid (thyroxine intravenously or desiccated thyroid by mouth) containing this quantity of iodine would not elevate the metabolism as much as the anterior lobe extracts. This suggested that thyroglobulin, as secreted by the gland, was much more effective than administered thyroid products. A study was undertaken to compare the rise in metabolism when thyroglobulin was administered subcutaneously and by stomach tube.

Five dogs trained for metabolic tests were employed. Each animal received a series of subcutaneous injections and later a series of oral administrations or, in some cases, the order was reversed. It was found that a much greater effect was produced by subcutaneous injection, the results being similar in all five dogs. Since the thyroglobulin was not pure, it is possible that some of the calorogenic effect after subcutaneous administration was due to foreign protein. The crude preparation is 4 to 5 times more active by this route. These studies indicate that the thyroid hormone, similar to many others, loses considerable activity in the alimentary canal.

Experimental diabetes treated by x-ray applied to the pituitary and adrenal regions. B. O. BARNES, W. L. CULPEPPER (by invitation) and J. H. HUTTON (by invitation). Department of Physiology, University of Chicago and Illinois Central Hospital.

Following the observation of one of us that some clinical cases of diabetes and hypertension were improved by x-ray treatments, a study was undertaken to see if experimental diabetes might be similarly influenced. All treatments have been given at the Illinois Central Hospital with the same x-ray equipment which was used before. Thus far both the pituitary and the adrenal regions have been x-rayed.

The procedure has been to remove the pancreas, and establish the animal's insulin requirement on a diet rich in carbohydrates. After a suitable control period, x-ray treatments were given at intervals. The results, to date, have been encouraging in that a considerable reduction of insulin was possible. Six dogs have been used, two of which were followed for many months. They showed no tendency to lose the benefit of the treatments during the period of observation. Further work is in progress to

determine the optimum dose and frequency of irradiation, and to analyze the nature of the improvement.

Implantation of dog pituitaries into immature rats. B. O. BARNES and H. M. FOWLER (by invitation). Department of Physiology, University of Chicago. (Read by title.)

In a previous report (Barnes and Bueno) it was found that bovine anterior pituitary extracts might stimulate the ovaries of the dog although they had little effect on immature rat ovaries. It seemed possible that the dog's own pituitary might be complementary to the beef extract. A total of 79 immature female rats has been used in the present study. The experiments were started when they were 21 days of age and they were autopsied five days later. Implantation of one adult male rat pituitary caused the usual stimulation of the ovaries, increase in weight of the uterus, and opening of the vaginal orifice. Untreated controls, controls injected with saline, or animals receiving beef pituitary extracts failed to show any change. Implantation of one or two male dog pituitaries into an immature rat caused no macroscopic change in the ovaries or uterus, and the vaginal orifice did not open. Furthermore, negative results were also obtained when in addition to the implantation of dog pituitaries, beef pituitary extract was administered daily. Further work is being done to see if the dog pituitary does not store gonadotropic hormone or if the rat is refractory to dog pituitary hormone.

The effect of splanchnic resection on gastric motility in man. LOUIS E. BARBERON (by invitation), GEORGE M. CURTIS and WILLIAM T. HAVERFIELD (by invitation). Department of Medical and Surgical Research, Ohio State University.

Observations on gastric motility were made on a patient before and after bilateral splanchnic resection. As a preliminary measure, a barium coated balloon was swallowed and its position in the stomach verified by means of the fluoroscope and x-ray. The exact length of rubber tubing from this position to the incisor teeth was measured and kept constant throughout the observations. A condom balloon, attached to a bromoform manometer by a rubber tube, was swallowed and inflated to a pressure of 2 cc. of bromoform at the beginning of each experiment. Gastric motility was then recorded on a kymograph. The observations were made in the morning, the patient receiving no food after the preceding evening meal. The usual duration of each experiment was five hours. Fifteen preoperative control observations were made. Bilateral splanchnic resection was performed by the super-diaphragmatic approach through the posterior mediastinum. The splanchnic nerves were identified, and large segments of each resected. The convalescence was uneventful. Twenty-one experiments were made after the left, and fifteen after the right splanchnic resection. The results indicate that following bilateral splanchnic resection the duration of the periods of activity is definitely increased. Although severe contractions were frequently observed, the subject at no time experienced symptoms suggestive of hunger.

Concurrent assay of three hormones from anterior pituitaries of seven age and sex groups of cattle. ROBERT W. BATES (by invitation), OSCAR RIDDLE and ERNEST L. LAHR (by invitation). Station for Experimental Evolution, Carnegie Institution.

Anterior pituitaries from seven groups of cattle were collected, extracted and all fractions assayed upon immature doves for their prolactin, F.S.H. and thyrotropic content. The seven types were as follows: embryos, calves, adult bulls, adult steers, cows not pregnant, cows in early pregnancy and cows in late pregnancy. Weight increase of crop-glands, testes and thyroids was the basis of assay. The anterior pituitaries of all seven groups were found to contain not strikingly dissimilar quantities of all three hormones when comparison was made on the basis of weight of fresh tissue. The prolactin content of embryo pituitaries, per gram of tissue, was three times that of other pituitaries with the exception of the glands from pregnant cows. The greatest F.S.H. content was found in glands from cows in early pregnancy and the least amount in the pituitaries of the male castrates. The thyrotropic content was greatest in pituitaries from cows in early pregnancy and least in glands from embryos and steers.

Effect of temperature changes on the circulation. H. C. BAZETT, J. C. SCOTT (by invitation), M. E. MAXFIELD (by invitation) and M. D. BLITHE (by invitation). Departments of Physiology, University of Pennsylvania and Hahnemann Medical College.

On 4 subjects in baths measurements were made of oxygen consumption, cardiac output by acetylene, pulse rate, blood pressures—by optical method previously described—pulse wave velocities (from the heart to subclavian, subclavian to femoral, subclavian to brachial, and femoral to dorsalis pedis), and rectal temperatures (recorded electrically). The effective arteriolar resistance was calculated from the ratio of mean pressure to cardiac index, on the assumption that a basal CI of 2.2 is associated in the young subject with a mean pressure of 73 mm.

In neutral baths, in spite of the supposed effects of hydrostatic pressure, cardiac output values were normal; the blood pressures were on the low side of the normal range. Cooling the bath induced usually an immediate reflex increase in oxygen consumption, with a tendency to shiver, which rapidly subsided. At this later stage the systolic pressure was always, diastolic and pulse pressure usually, increased, while cardiac output might be increased or decreased. The cooling was usually insufficient to cause continuous shivering, which would have interfered with the records, and a decreased oxygen consumption usually accompanied a decreased cardiac output. Peripheral resistance was increased particularly with mild cold before the onset of later shivering.

On warming, marked discomfort accompanied by hyperpnea was associated with the period 1 of rapidly rising temperature; the subject was at first more comfortable when the rectal temperature ceased to rise (period 2), but later with continuing dehydration (up to 5.5 per cent of body weight) might again become distressed with marked restlessness (period 3). In periods 1 and 2 systolic pressure was increased, diastolic pressure little changed, pulse pressure increased, though the stroke volume was reduced; the increased pulse rate raised the cardiac output, and peripheral resistance was lowered, yet the central pulse wave velocities were more rapid. There appeared to be a reciprocal relationship between the reactions of the larger and smaller arteries. In period 3 the cardiac output was reduced and peripheral resistance increased. Calculation of stroke volume from the blood pressures sometimes gave good agreement with acetylene values, at other times marked discrepancies; this relation is being investigated further.

Alteration in the response of mammalian muscle to electrical stimulation of nerve as a result of changes in pressure. JOHN W. BEAN. Department of Physiology, University of Michigan, Ann Arbor.

Dogs anesthetized with morphine and urethane were exposed to various pressures of oxygen and of air up to 60 pounds per square inch. Blood pressure, pulmonary ventilation and response of the tibialis anticus muscle to intermittent electrical stimuli, applied to the central end of the sectioned popliteal nerve, intact peroneal nerve, peripheral end of the sectioned peroneal nerve, and directly to the muscle, were recorded. Increasing the pressure above atmospheric, gave rise to a decrease and cessation of the muscle response elicited by stimulation of the central end of the popliteal nerve, intact, and peripheral end of the sectioned peroneal nerve but not when elicited by direct stimulation of the muscle itself. This cessation frequently persisted throughout prolonged periods of exposure to the increased pressure. In some instances there was a gradual return of the response during prolonged exposure. Decompression was attended by a return of the response in a graded fashion. The relative suddenness of onset of the cessation effect and the low pressures by which it was sometimes produced (2 pounds per square inch), suggested that it might be due to some artefact. In experiments in which the central end of the popliteal nerve and the intact peroneal nerve were stimulated, cold block applied to the vagi brought about a return of the muscle response which had previously been stopped by a maintained pressure; removal of the cold block resulted in cessation again. These experiments support the belief that the cessation phenomenon is not an artefact. These cold block effects were not produced in those experiments in which the peripheral end of the sectioned peroneal nerve was stimulated or in those of direct muscle stimulation. The cessation phenomenon was also observed in experiments on frog muscle-nerve preparations, but not so consistently. The cessation in the frog experiments was attended by some contracture which appeared in less marked degree in the mammalian experiments.

Ketosis in normal and Eck fistula dogs. JAMES M. BEAZELL (introduced by Lathan A. Crandall, Jr.). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Illinois.

Although various studies have indicated that alterations in liver function may influence the development of ketosis, the evidence at hand is meagre. Therefore, Eck fistula dogs and normal dogs were compared as to urinary ketone excretion during: 1, fasting, and 2, a diet of olive oil exclusively.

In two Eck fistula and four normal dogs twenty-four hour urinary ketone excretions were determined during fasts ranging from seven to sixteen days. None of the normal dogs showed an increased ketonuria (as compared with the pre-fasting, normal diet period) during this time. Increased ketone output, however, was present in both the Eck fistula dogs.

In four Ecks and four normals placed on 10 cc. olive oil per kilo (no other food) for periods of ten to twenty days, an increased excretion of ketone bodies was present in all. The ketonuria appeared within three days in the Eck fistula animals, and not until the eighth to the tenth day in the normals. The amount excreted by the normal animals was much greater than that eliminated by the dogs with Eck fistulae.

It seems probable that decreased carbohydrate storage may account for the earlier appearance of ketosis in Eck fistula dogs, while the decreased amount eliminated by these animals suggests that the liver plays a major part in ketone body formation.

A statistical evaluation of the linear vs. the exponential (surface area) method of predicting metabolism. JOSEPH BERKSON, WALTER M. BOOTHBY and HALBERT L. DUNN. Section on Biometry and Medical Statistics and the Section on Clinical Metabolism, the Mayo Clinic, Rochester, Minn.

A statistical inquiry has been made to determine whether the heat production of normal human subjects is better predicted from stature, body weight and age by 1, a linear type of equation (Harris and Benedict), or 2, an exponential type of equation (surface area, Du Bois). The basic data used comprise a series of observations made at the Mayo Clinic (641 males, 776 females) and the series of Harris and Benedict (136 males, 103 females). Several other smaller series were also examined.

Using as criterion, the magnitude of the root-mean-square deviation of predicted from observed heat production, we found that the linear formula does not give the better prediction. The contrary conclusion of Harris and Benedict is due to the omission by them of the *age* variable in calculating the heat production from "surface area" while including it in the predictions made from the linear formula.

The effects of diets low in choline on normal rats and depancreatized dogs.

C. H. BEST, M. E. HUNTSMAN (by invitation), J. H. RIDOUT (by invitation) and F. G. YOUNG (by invitation). Department of Physiology and the School of Hygiene, University of Toronto.

It has been reported previously that certain diets containing an extremely small amount of choline, but adequate in other respects, have no "lipotropic" effect when fed to rats whose livers have been made fatty by fat feeding. The term "lipotropic" has been coined to describe the action of certain substances in preventing the accumulation of fat in normal livers and in reducing the fat content of fatty livers. When normal rats are placed on a diet containing only small amounts of "lipotropic" substance there is a rapid accumulation of neutral fat in the liver. This is completely prevented by the addition of small amounts of choline to the diet. Certain other diets which also contain only very small amounts of choline may still exert an appreciable "lipotropic" effect. This may be due to the presence of betaine, which is known to exert this effect, or to other "lipotropic" substances as yet unknown. At present the amount of such material which is contained in a diet can be determined only by investigating its effect on the rate of accumulation or disappearance of liver fat.

The deposition of fat in the livers of depancreatized dogs with and without insulin is rapid and extensive when diets low in "lipotropic" substances are used. This accumulation of fat in some cases may be accompanied by an increased sensitivity to insulin and a diminished sugar excretion. These results suggest that, hitherto unrecognized, dietary factors may rather promptly affect the intensity of glucosuria in pancreatic diabetes in dogs, probably by altering the rate of formation of sugar by the liver.

Further studies on the ketogenic hormone of the anterior pituitary. PETER T. BLACK (by invitation), J. B. COLLIP and D. L. THOMSON. Department of Biochemistry and Pathological Chemistry, McGill University. Evidence is presented to show that the ketogenic principle is distinct from the growth, thyrotropic, adrenotropic and maturity hormones. It is active in adrenalectomized or thyroidectomized animals.

Chronic treatment of rats with ketogenic extracts render them resistant to its action, even when they are placed on a high fat diet. Phlorhizin ketosis is greatly diminished. The sera of these animals, when injected into otherwise normal animals, render them resistant to the ketogenic principle.

Observations on the development of electrical excitation in nerve. E. A. BLAIR (by invitation) and JOSEPH ERLANGER. Physiological Department, Washington University School of Medicine.

Spontaneous variations in the response time of alpha axons to rheobasic currents have previously been demonstrated with the cathode ray oscillograph. This play of response time is referred to spontaneous changes in irritability occurring during a plateau of excitation lasting ordinarily nearly 1.5 ms. The curve of excitation as indicated by the response pattern to rheobasic currents does not agree temporally with the curve for subrheobasic currents as indicated by the shock voltage method. This discrepancy is referable to the fact that the time required for the exciting effect of the shock to sum with that produced by a constant current to the point of stimulation varies temporally with imposed conditions. Thus in normal nerve excitation following a shock reaches maximum after a delay of 0.3 ms. and in nerve under threshold polarization delay is about doubled and the rate of decline decreased. When these factors are discounted the plateau becomes an expression of the relatively steady state of excitation indicated by the play of response time. The end of the plateau as represented by the angle in irritability curves determined by the shock method and by the longest response times has a temperature coefficient much higher than that of the beginning of the plateau similarly measured.

This interpretation is substantiated by the fact that the voltage of a long rectangular current required for threshold when superimposed on one 80 per cent of rheobase is not materially changed by separating the starts 0.5 ms. With further separation the irritability thus determined falls off abruptly along a decrementing curve.

The fact that the increasing excitation following, and during, a shock or a constant current can be modified by an opposing potential (anodal shock) indicates that the decrease in threshold is referable to a change of potential at the responding locus. This electrical change in the latter case reaches a maintained, effectively stable maximum at 1.6 ms. The delayed onset of the depression in irritability (3.0 ms.) that terminates this state is assigned to the time required for the passage of ions ($K^+?$) by diffusion through the Nernst membrane.

Thermal changes in mechanical and chemical obstruction to the circulation of the extremity. B. H. BLOCKSM, JR. (by invitation), C. B. HUGGINS and HARWELL WILSON (by invitation). University of Chicago.

This is a study of reactive hyperemia with thermocouples. The subjects used were man, dog and rabbit. Thermocouples were placed at analogous convenient points on both extremities; in rabbits the couples

were placed in the medullary cavities of the long bones as well as intramuscularly and subcutaneously. One extremity was constricted with a tourniquet and the temperature followed during constriction and for a period following release of the constriction while the other extremity was used as a control. In some experiments additional couples were placed on the arm or the sternum or just above the tourniquet on the experimental extremity. In some cases the intra-arterial injection of epinephrine in the experimental extremity was substituted for mechanical constriction.

Plotted curves of the results showed: 1, a fall in the temperature of the experimental leg during constriction; 2, hyperthermia in the experimental leg following release of the tourniquet; 3, participation of all the deeper structures of the leg in this hyperthermia, the bone marrow showing the greatest response; 4, a definite fall in temperature in all the control areas following release of the tourniquet; 5, a fall in temperature in the experimental extremity with intra-arterial epinephrine almost exactly similar to the fall when mechanical constriction was employed; recovery of the extremity in this case was not followed by hyperthermia.

On hyperglycemic effect of tobacco smoking. EPHRAIM B. BOLDYREFF (introduced by W. R. Bloor). Pavlov Physiological Institute, Battle Creek Sanitarium.

During the summer of 1929 it was demonstrated by the author in dogs with permanent tracheal fistulae that pipe smoking of Virginia cutleaf tobacco induces appreciable although temporary rise of blood sugar. These findings were subsequently verified in experiments with cigarette smoking in tracheotomized dogs and normal human subjects. Common and denicotinized cigarettes being used. Again a noticeable hyperglycemic action was observed in all cases. Later the hyperglycemic effect of smoking was reported also by Lundberg and Lundberg (1931), and Haggard and Greenberg (1934). These investigators, however, attribute the rise of blood sugar caused by smoking to nicotine. Their conclusions cannot be applied to the author's findings since the increase of blood sugar was also observed in the case of smoking of denicotinized tobacco. Besides such views are contradictory to previously reported data of Macleod and Dolly (1905), and Foglia (1931). Therefore to elucidate further this point a series of experiments with administration of the pure alkaloid and nicotinic acid (oxidation product) was carried out in dogs, turtles, and human subjects. It was found that nicotine was without any effect on blood sugar (dogs, dose: 4 mgm.). On the other hand nicotinic acid exerted a mild hypoglycemic action (turtles, 10 mgm.; dogs, 5-15 mgm.; man, 1.5 mgm.). In all cases an increase of muscular sensitivity, decrease of body temperature, and slowing of heart beat, were noted. The hypoglycemic action of nicotinic acid was most pronounced in turtles. Thus, it seems that the increase of blood sugar caused by tobacco smoking should be attributed to other factors than nicotine or its oxidation product.

Gastric and intestinal mucus—its properties and physiological importance.

W. N. BOLDYREFF (introduced by A. C. Ivy). Pavlov Physiological Laboratory of the Battle Creek Sanitarium.

The author has studied the gastric and intestinal mucus, the mucus from the respiratory tract and some vegetable mucus (okra, psylla and others). In none of these cases did he find the pure mucus to possess any independent ferment of its own.

All of the animal and vegetable mucus studied by the author had a slightly alkaline reaction to litmus, was colloidal semi-transparent and colorless (with the exception of the intestinal mucus which is not transparent and is slightly yellow), gelatinous, viscous, heavier than water and capable of adsorbing various substances in large amounts.

The author's experiments have shown that mucus is capable of adsorbing acids, alkalies and salts; it also adsorbs a vast amount of ferment and carries them along and later sets them loose whereby their destructive action may be developed in remote places.

In the small intestine the quantity of mucus is the greatest the nearest to the colon.

Large amounts of mucus hinder normal digestion.

Besides the inorganic and organic soluble substances mucus is also capable of holding in itself solid objects and even live organisms (bacteria). Possibly, in this manner mucus is a temporary protection against some infections. But later it may become a source of them.

The author has found that insulin possesses a very pronounced secretagogue action on the gastric mucosa for the secretion of mucus. By this means one can easily obtain pure mucus free of any ferment.

Gastric mucin (Fogelson-Stearns) now used in the clinic contains a large amount of pepsin and so-called rennin. Sterilization removes its effect.

This work is being continued.

A clinical standard for metabolic heat production. WALTER M. BOOTHBY, HALBERT L. DUNN and JOSEPH BERKSON. Section on Clinical Metabolism and the Section on Biometry and Medical Statistics, the Mayo Clinic, Rochester, Minn.

A biometric analysis was made of the observations on 641 male and 776 female normal human subjects with respect to heat production, age, stature, and body weight. A smoothed curve was established representing the mean values of heat production, in calories per square meter per hour, at various ages, over the entire life span, and for both sexes separately. From these mean values and a smooth curve for the standard deviations, a probability nomogram was constructed. Given the sex and age of an individual, and a determination of the heat production in calories per square meter per hour, one can, with a single setting of a rule on the nomogram, determine 1, the B.M.R. as customarily expressed, that is, as per cent deviation from mean; 2, the actual probability of normality, in the sense that one reads the number of persons out of 100 normal individuals who would have so large a deviation from the mean, as does the subject under consideration.

The motility of the dog's colon following ileostomy.¹ E. L. BORKON (by invitation), R. D. TEMPLETON (by invitation) and H. C. LAWSON. Department of Physiology, University of Chicago and Department of Physiology, Loyola University, School of Medicine.

This study of the motility of the dog's colon following ileostomy was controlled by tracings recorded for three months previous to the operation. Following a cecostomy the activity of the colon was obtained in the trained unanesthetized animal with two tandem systems of three balloons each.

¹ Aided in part by a grant from the Henry Strong Denison Medical Research Foundation.

One set was inserted through the cecostomy and the other by way of the anus. During the standardizing period thirty uninterrupted four hundred minute experiments were run.

At the conclusion of the control period, the large bowel was isolated by an ileostomy and the animal allowed a short time for convalescence before resuming the work. Fifteen days after this second operation, the investigation was renewed using the same procedure as practiced before the colon isolation.

To show the relation of the active to the quiet periods of motility, a method of tabulation was developed in which time was the only element considered. By this method graphs were obtained which showed the spatial relationship of the activity of each segment at any given instant. Thus the number of minutes of motility for a given period of time in any part of the colon could be represented graphically in sharp contrast to the number of minutes of inactivity.

The results revealed that isolating the colon did not markedly alter the motility, i.e., the same general pattern showing periods of activity alternating with periods of relative inactivity continued. Prior to the ileostomy the active periods were more irregular and frequently separated from one another by indistinct low-grade activity. After the ileostomy the relatively inactive periods became distinctly more quiet. This tended to accentuate the essential periodicity of the motility. A decreased quantity of motility was seen during the first month following ileostomy. Thereafter (and up to the present, over 900 days since operation) the quantity of gut motility became comparable to that preceding operation. With the isolated colon preparation, it was necessary to inflate the balloons more than in the control period if motility of comparable height was to be recorded. Tone changes were conspicuously absent except at times of gut contraction.

The influence of temperature on the vagal inhibitory mechanism of the turtle heart. T. E. BOYD and DOMINIC BAIMA (by invitation). Department of Physiology and Pharmacology, Loyola University School of Medicine, Chicago.

The turtle vagus was stimulated by amplified current from a photoelectric cell, with a rotating disc interrupter giving nearly rectangular shocks of 4σ duration. The stimulated part of the nerve was left at room temperature, 18 to 22°C.

The optimal frequency of stimulation varies with the temperature of the heart. At 14 to 18°, the optimum is 6 to 8 per second, as several earlier workers have found. When the heart is bathed in Ringer at 6 to 8°, the chronotropic and inotropic effects are reduced for all frequencies, and differences are not so evident. At 24° or higher, stimulation at 30 per second is more effective than at 10. Extreme heat (40 to 43°) may produce a nicotine-like block.

When the vagus nerve (freshly dissected) is locally cooled between the point of stimulation and the heart, the inotropic effect of a single shock persists unchanged to -4°, unless the nerve is frozen. With repetitive stimulation, the effect begins to diminish at a higher temperature, depending on the frequency. The chronotropic effect may be lost at 0° or higher, while the inotropic always remains in some degree until the nerve is frozen.

With local cooling and continued stimulation at a high frequency, the ventral shows early "escape." It may then be brought into standstill again either by allowing the nerve to warm slightly or by shifting to a lower frequency of stimulation.

Effect of dorsal root stimulation on the properties of the muscles of blood vessels. E. BOZLER. Johnson Foundation, University of Pennsylvania.

An attempt is made to analyse the changes which produce the relaxation of the blood vessels after stimulation of vasodilators. One hind leg of a frog is perfused with Ringer. A sensitive flow meter, based on the principle of the Pitot tube, has been used to record the reactions of the blood vessels conveniently and without distortion. Contraction is produced by stimulating the sympathetic trunk; inhibition by stimulating the dorsal roots. It is found that stimulating the dorsal roots does not suppress nor even diminish the magnitude of the response to sympathetic stimulation. However, if the dorsal root stimulation is applied after the stimulation of the sympathetic, the relaxation is greatly accelerated.

The viscous properties have been studied by suddenly raising the perfusion pressure and observing the change of flow. Following the pressure change the blood vessels widen only slowly, indicating a large viscous resistance in the walls of the vessels. Stimulation of the dorsal roots accelerates this process very markedly, which is evidence that the viscous properties of the muscles of the blood vessels have been changed.

Comparison of changes in the pH of arterial blood and saliva during variations of pulmonary ventilation. CHARLES R. BRASSFIELD (introduced by Robert Gesell). Department of Physiology, University of Michigan, Ann Arbor.

pH determinations were made by means of the glass electrode on blood samples taken from the femoral artery and on saliva samples taken from the submaxillary gland of dogs. Continuous pH measurements also made by mounting a glass electrode in the flowing saliva elicited by a continuous injection of pilocarpine.

Changes in pulmonary ventilation were produced by the administration of low oxygen, high carbon dioxide, sodium bicarbonate, and sodium cyanide.

Lowering the oxygen tension of the inspired air caused an increase in the pH of arterial blood with a decrease toward the resting level if the administration were prolonged. The resting level was quickly reached upon returning the animal to room air. The pH of saliva initially increased and then gradually decreased below the preadministration level. If the administration were prolonged a second slight increase might occur and in all cases a marked increase took place upon returning the animal to room air. This increase was usually above the preadministration level and the pH frequently remained at this new level.

Increasing the carbon dioxide tension of the inspired air caused a decrease in the pH of blood which was very rapid during the first few seconds of the administration. There was a rapid return to the resting level when the animal was given room air. The pH of saliva was decreased sharply at first, then gradually increased throughout the administration. If the procedure were prolonged this gradual increase reached or exceeded the resting level. On returning the animal to room air a marked increase in pH above the preadministration level was noted.

Intravenous injection of sodium bicarbonate caused an increase in both the blood and saliva pH followed by a gradual diminution toward the resting level.

Injection of sodium cyanide caused an increase in both blood and saliva pH followed by a return to resting levels and in the case of saliva a decrease below the preinjection level has been noted.

Effects of saline and other solutions on estrus and survival after adrenalectomy.

S. W. BRITTON and R. KLINE (by invitation). Physiological Laboratory, University of Virginia Medical School.

A number of different salt solutions have been used in testing the estrual activity and period of survival of adrenalectomized rats. Solutions similar to those used and apparently found effective by Rubin and Krick were included in the series. All the solutions used in our experiments were without significant effect, however, on the usual anestrous condition observed after adrenalectomy, as well as on the survival period. There was also no notable amelioration of the usual symptoms of adrenal insufficiency following administration of saline solutions to both rats and cats. Glucose injections brought about striking and repeated recovery from the terminal convulsive seizures of adrenal insufficiency, however, although there was only slight prolongation of the survival period. The evidence favors the carbohydrate theory of cortico-adrenal function.

The influence of partial and complete adrenalectomy on reproduction and lactation. S. W. BRITTON and R. KLINE (by invitation). Physiological Laboratory, University of Virginia Medical School. (Read by title.)

Even partial adrenal removal seriously affects the course of pregnancy and lactation in cats and rats. Unilateral adrenalectomy of pregnant cats is frequently followed by abortion, or death of the kittens occurs a day or two after birth through failure of the mother to lactate and nurse the young. Removal of one adrenal and half the other in cats results in abortion, although the mother survives in apparently normal health. Partial adrenalectomy of pregnant rats is frequently but not invariably serious, apparently dependent on the amount of accessory cortico-adrenal tissue present in this animal. Unilateral adrenalectomy of the rat, however, often results in prolonged or permanent sterility. Complete protection of the normal reproductive and lactating functions of the rat appears to be afforded after adrenalectomy by the administration of cortico-adrenal extract.

Synaptic transmission in a sympathetic ganglion. D. W. BRONK, R. J. PUMPHREY (by invitation) and J. P. HERVEY (by invitation). Johnson Foundation, University of Pennsylvania.

The object of the experiments here described is to determine the capacity of a sympathetic ganglion to transmit impulses of various frequencies and to study the effect of previous activity on the properties of the ganglion. The results are of interest in connection with the general problem of synaptic transmission.

We have stimulated by means of a thyratron stimulator preganglionic fibres to the stellate ganglion of a cat and have recorded with an oscillograph the postganglionic impulses in the inferior cardiac nerve. Confirming the earlier work of Bronk, Solandt and Tower we find that at low frequencies of

stimulation each preganglionic volley sets up a single postganglionic volley and that in addition there is a certain amount of temporal dispersion of impulses in the ganglion due to repetitive firing, different latencies or both these effects.

At frequencies above about 80 per second only the first few preganglionic impulses are transmitted as definite postganglionic volleys. These volleys rapidly decrease in size and disappear, apparently indicative of ganglionic block. That this is not the complete explanation of the phenomenon however is shown by the fact that stimulation of the preganglionic fibers at a frequency of 200 per second produces continued cardiac acceleration for some minutes. It is more probable therefore that the rapid stimulation so alters the properties of the ganglion that there is a marked temporal dispersion with a consequent smoothing out of the postganglionic activity. This was established as the correct explanation by cutting down the post-ganglionic trunk to a few fibers. The action potential record then showed a complete scattering of impulses after the first few synchronized volleys. This is presumably due to changes in synaptic latencies resulting from previous activity.

By sending through the ganglion a burst of impulses for two seconds followed at various intervals by a second such burst the recovery of the ganglion's capacity to transmit synchronized volleys of impulses has been followed. There is a rapid recovery of the capacity for transmitting the first ten or fifteen preganglionic volleys and a much slower recovery of the capacity for sustained transmission of this type.

Studies on the neural basis of ovulation in the rabbit.¹ CHANDLER Mc.C. BROOKS. Department of Physiology, Johns Hopkins University, School of Medicine.

Each of five female rabbits from which both sympathetic chains had been completely removed ovulated normally following coitus. Six animals without abdominal sympathetic chains, five from which the stellate ganglia had been removed and four with abdominal chains, stellates and upper thoracic chains extirpated, not only repeatedly ovulated but have successfully borne and reared litters of normal size. In the female rabbit a sympathetic nerve supply to the hypophysis is not essential for its gonadotropic function nor, in this species, is a sympathetic innervation of a mammary gland necessary for its lactation.

Ovulation occurred normally following coitus in rabbits whose sacral cords had been removed. Even when this procedure was supplemented by complete abdominal sympathectomy, hysterectomy and extirpation of the proximal half of the vagina, coitus was still followed by ovulation. All such preparations exhibited full anesthesia of the genitalia and of the skin surrounding the vulva. Specific genital stimulation is therefore not an indispensable factor in the induction of ovulation. Females whose hindlegs had been completely paralyzed by lumbar or thoracic cord section failed to ovulate, though males mounted them, intromitted, ejaculated and fell off when the paralyzed animal was placed in such an attitude that this was mechanically possible. Such observations suggest that the female must coöperate if the sexual activity is to be effective in inducing ovulation.

¹ Aided by a grant (to Dr. Philip Bard) from the Committee for Research in Problems of Sex, National Research Council.

Active coöperation is in turn probably dependent on sexual "excitement." This interpretation is supported by finding *a*, that females with deafferented genitalia do not ovulate after being mounted unless they permit intromission by making a specific postural adjustment, and *b*, that when one female mounts another, ovulation follows in the former only if, after executing male-like copulatory movements, she falls off as do males on ejaculation. Confirmation of the report that the mounted individual of such a pair later ovulates could not be obtained.

Compensation for blood loss in chronic spinal cats. CHANDLER McC BROOKS. Department of Physiology, Johns Hopkins School of Medicine. (Read by title.)

Chronic spinal cats, in which all connections between higher centers and the thoraco-lumbar sympathetic outflow have been severed by a sixth cervical transection of the cord, compensate for low arterial pressure induced by severe blood loss less effectively but in practically the same ways as do normal animals. Following hemorrhage, if the vagi are cut the heart rate is immediately accelerated, the denervated nictitating membrane contracts, the blood elots more quickly, blood sugar rises, vasoconstriction occurs, and the arterial pressure returns approximately to normal and frequently rises momentarily above the prehemorrhage level. The occurrence of vasoconstriction following hemorrhage was demonstrated by the greater decrease of volume in an innervated as compared with a denervated limb and by the increased resistance to the flow of a perfusing fluid through a leg entirely isolated, save for its nervous connections, from the rest of the body. The initiation of this compensatory activity of the sympathico-adrenal system depends on some agency other than the carotid sinus and depressor nerve mechanisms.

Complete removal of the sympathetic chains greatly reduces the ability of a spinal vagotomized animal quickly to restore its arterial pressure to normal after hemorrhage. The denervated adrenal glands of such preparations, however, are capable of responding, probably as a result of direct asphyxial stimulation to low arterial pressure. After adrenalectomy hemorrhage fails to elicit contraction of the denervated nictitating membrane, cardiac acceleration, vasoconstriction and significant changes in blood sugar and blood clotting time. The slow rise in arterial pressure which occurs after hemorrhage in completely sympathectomized cats with vagi cut and adrenals out may be explained on the basis of a restoration of blood volume by an inflow of tissue fluid.

The effect of fibro-deutero-proteose on the blood. CLYDE BROOKS. Departments of Physiology and Pharmacology, Louisiana State University Medical Center, New Orleans. (Read by title.)

Ox-blood fibrin was washed; digested with HCl and pepsin; the first fraction, precipitated with half-saturated ammonium sulphate, was rejected; the second fraction, precipitated with full-saturated ammonium sulphate, was isolated and prepared, in sterile solution, for administration to animals, normal persons, and to patients.

The results of a single adequate injection are: a leucocytosis, beginning without any period of preliminary depression, and reaching the crest in five or six hours, after which there is a gradual fall, which does not reach the previous normal level till next day, or later.

During this leucocytosis the granulocytes are the first to rise, followed by the lymphocytes, and finally by the monocytes. Likewise, when the fall sets in, the granulocytes are first to decline, followed by the lymphocytes, and lastly by the monocytes.

Also during the leucocytosis there is a "shift to the left" or increase in stabs and juvenile forms which do not decline when the fall in leucocytosis sets in; but which take a much longer time to return to the former normal level.

These changes in the leucocyte count are similar to those which occur in certain acute infections, or in instances of sudden parenteral entrance into the body of infectious material, or of other foreign protein.

The liberation of energy in the contracture and simple twitch. DUGALD E. S. BROWN. University and Bellevue Hospital Medical College, New York University.

The consensus of opinion of recent investigators favors the view that a contracture differs from a motor contraction chiefly in the rate of release of the chemical substances causing contraction. In the pressure contracture, the rate of release of these agents is contingent only upon the maintenance of pressure, and tends to be terminated at any time by an abrupt decompression. Accordingly by decompressing the muscle at various times after compression it is possible to stop the further liberation of energy, after a certain quantity has been formed. This allows one to ascertain the energy liberated at various times during the contracture and also the form of the contraction after decompression when no further energy is being mobilized. Experiments performed in this way show that if the muscle is decompressed when only five per cent of the expected contracture tension has had time to develop, the subsequent contraction is similar in rate and form to a simple isometric twitch. This contraction is not due to a stimulation of the muscle since it is unaccompanied by a propagated action potential. It is believed that during the brief period of compression a quantity of chemical energy is liberated and that after decompression this fraction causes contraction. The situation is therefore similar to that in the twitch where heat measurements show that a rapid burst of energy occurs at the onset of contraction. The similarity in the form of the two contractions bears out this interpretation. In contrast to this, the pressure contracture appears to depend on a continuous liberation of chemical components.

Alterations in respiratory rate produced by electrical excitation of the cerebral cortex of dogs. P. C. BUCY and T. J. CASE (by invitation). Division of Neurology and Neurosurgery of the University of Chicago.

In dogs, under light ether anesthesia, faradic stimulation of a small area of the cerebral cortex, about 5 mm. in diameter, lying oral to the anterior end of the coronal suture produces marked diminution in the respiratory rate. The amount of diminution varies with several factors the most important of which are the depth of anesthesia and the intensity of the stimulus. With very strong stimulation apnea can be produced for short periods of time. Diminution in respiratory rate cannot be obtained from neighboring portions of the cortex except with stimuli of great intensity and the results are interpreted as due to spread of the electrical effect to the area described. Apnea has not been obtained from any other portion of

the cortex. Nor can any comparable effects be obtained from stimulation of neighboring non-nervous tissues, i.e., the dura, temporal muscle and orbital tissues. The alterations in rate are associated with a variety of changes in amplitude of respiratory excursion. The amplitude may be unchanged, increased or decreased. Alterations in blood pressure are minimal and inconstant. The effect on respiratory rate is not influenced by extirpation of the sigmoid gyrus, incising the cortex and subcortical white matter cephalad to or superior to the effective area but is destroyed by a deep incision posterior to the area. The effect is not altered by section of the vagi or phrenic nerves.

Dr. S. W. Ranson and his co-workers have obtained comparable results from stimulation of the pre-optic region (paper in press). However, as the course of the efferent fibers from the cortical area concerned in our experiments has not been determined no correlation between their results and those reported here is as yet possible.

Study on specific dynamic action following removal of various endocrine glands of dogs. J. G. BUENO (by invitation), B. O. BARNES and J. M. ROGOFF.¹ Department of Physiology, University of Chicago. (Read by title.)

An influence on specific dynamic action has been attributed to some of the endocrine glands. We have made some observations on this question, using dogs which were being employed for other studies. Twenty-one animals trained for metabolic determinations were employed. These include 3 normal, 4 thyroidectomized, 4 hypophysectomized, 1 hypophysectomized and depancreatized, 3 with suppression of epinephrine secretion, 2 depancreatized, and 4 with epinephrine secretion suppressed in addition to pancreatectomy. In all cases the B.M.R. was determined just before feeding 200 grams of meat and a determination of the metabolic rate was made again after 2, 3, 4, 5, or 6 hours. An average of 10 determinations was made on each dog and the time of the second observation varied on different days.

Several determinations on the same animal reveal that considerable variation may occur from day to day. Individual variations are encountered among the animals in a given group. Our results agree with the report of others that thyroidectomy or hypophysectomy does not abolish specific dynamic action. If any change occurred, it was slight and not significant in the number of animals used. Furthermore, the loss of the pancreas or the suppression of epinephrine secretion caused no noticeable change in the specific dynamic action of meat.

A comparison of the catalytic activity of the inorganic constituent of ash of different organs of the human. W. E. BURGE and O. S. ORTH (by invitation). Department of Physiology, University of Illinois, Urbana, Illinois. (Read by title.)

Portions of human liver, testis, pancreas, lung, plain, striated, and cardiac muscle, and kidney were incinerated at a dull red heat. The ash was first treated with hydrochloric acid, then the undissolved residue with nitric acid and evaporated to dryness over a water bath. One hundred milligrams of the ash of each tissue were added to 100 cc. portions

¹ Aided by a grant from the Commodore Beaumont Foundation.

of hydrogen peroxide in bottles, and the oxygen liberated in 72 hours was collected in burettes inverted in water. The liver liberated 680 cc., the testis 557 cc., pancreas 377 cc., lung 217 cc., plain muscle 208 cc., striated muscle 177 cc., cardiac muscle 153 cc., and the kidney 0 cc.

By comparing these figures it will be seen that the ash of the liver possessed the greatest catalytic activity as is indicated by the liberation of the largest amount of oxygen, the testis next, then the pancreas, lung, plain muscle, striated muscle, cardiac muscle, and the kidney.

It should be mentioned in this connection that the hydrogen peroxide as well as the ash solution was at pH 7.0. All the determinations were carried out at the same time under conditions of ordinary room temperature around 22°C. and since all the experiments were performed simultaneously no special precautions were taken to maintain the temperature absolutely constant. Checks were carried out, that is, bottles containing hydrogen peroxide but no ash to determine the amount of oxygen liberated from the hydrogen peroxide alone and the necessary corrections made. So far we have been unable to determine what in the ash is responsible for its catalytic activity.

The effect of daylight and darkness and the performance of physical work on the irritability and physical strength of the plant mimosa. W. E. BURGE and G. C. WICKWIRE (by invitation). Department of Physiology, University of Illinois, Urbana, Illinois.

The sensitive plant, *Mimosa pudica*,¹ was made to perform physical work by suspending a 400 mgm. weight from the leaves of this plant. When these leaves were stimulated they made a downward movement or dropped and during the succeeding 60 minutes, while returning to their original position, performed physical work by raising the weights. The leaves were made to perform work in this way for 30 days and at the end of this time they could do almost twice as much as the control leaves which had performed no work. It was also found that those leaves that had done no work fatigued much more easily than those that had performed work. Hence, it would seem that the performance of physical work within physiological limits has the same strengthening effect on the leaves of *Mimosa* as it has on animal muscles. Whether or not overworking the plant has a harmful effect as is the case with animal muscle is being studied.

Using threshold or minimal stimuli as a measure it was found contrary to observations reported in the literature that the irritability of *Mimosa* rose abruptly between daybreak and 10 a.m. from the low night time level, remained high during the day, and fell during the night. It was also found that if the plants were kept continuously illuminated, irritability remained at the high daytime-level, and if kept in darkness, irritability remained at the low night-time level. Keeping the plant in the darkness for long periods of time, a week or more, resulted in the complete loss of irritability and injury to the plant. The effect of keeping the plants continuously illuminated with the resulting continuous heightened irritability is also being studied.

¹ Our thanks are due Professor Fuller of the Botany Department for permitting us to use his *Mimosa* plants in this investigation.

A comparison of the rate of disintegration of nucleated and non-nucleated red blood cells, or red cells from anemic and normal animals and the effect of copper. W. E. BURGE and G. C. WICKWIRE (by invitation). Department of Physiology, University of Illinois, Urbana, Illinois. (Read by title.)

Small amounts of arterial blood were drawn under aseptic conditions from man, woman, mare, gelding, rabbit, dog, rat, mouse, turtle, and frog. After defibrination, hanging drops of blood were prepared in the animal's own serum, and the rate of disintegration of the red cells was studied with the use of a microscope. These experiments were carried out at ordinary room temperature around 22°C. and evaporation was prevented by keeping the hanging drop slides in a container saturated with water vapor.

It was found that the nucleated red cells of the frog and turtle disintegrated more rapidly than the non-nucleated cells of the mammals used. Of the mammals, the cells of man disintegrated least rapidly, horse next, then dog, and the red cells of the mouse disintegrated most rapidly.

It was also found that the red cells of rats with nutritional anemia disintegrated more rapidly than the red cells of the control animals. The addition of traces of copper sulphate decreased the rate of disintegration of the red cells in vitro and administration of traces of copper to rats with nutritional anemia decreased the rate of disintegration of the red cells in vivo. From these observations it would seem that copper, in addition to stimulating red blood cell formation in nutritional anemia also decreases the rate of disintegration of these cells. The effect on the rate of disintegration of the red blood cells of other inorganic elements which are claimed to be helpful in treating nutritional anemia is also being studied.

Relation of the vagi to hyperemia and erosions in the gastro-intestinal tract following parathyroidectomy. G. E. BURGET and WERNER ZELLER (by invitation). Department of Physiology, University of Oregon Medical School.

Both vagi were sectioned in the lower thorax in fifteen dogs. After five to fifteen days the thyroids and parathyroids were removed. In thirteen animals typical tetany followed in two to four days; two showed a more chronic type of deficiency. The survival period varied between two and ten days. In a few instances in this and in the control group when it seemed that nothing could be gained by allowing tetany to continue chloroform was administered and autopsy performed at once. Macroscopic examination of the gastro-intestinal tract showed no evidence of trauma or other pathology in ten animals. In the remaining five hyperemia of the pyloric canal occurred three times, hyperemia of the duodenum four times, hyperemia of the fundus of the stomach once and hyperemia of the jejunum once. In eight control animals (thyroid-parathyroidectomy, vagi intact) the survival period varied from two to six days. Hyperemia of greater or less extent together with occasional erosions of the mucosa were present in seven. One animal although it had tetany to a comparable degree with the other animals of this group, showed no evidence of trauma and no erosions of the mucosa.

These observations indicate that during parathyroid tetany a great increase in the flow of impulses over the vagi induces abnormal activity of the gastro-intestinal tract with traumatic effects.

Survival time and nutritional state of the gastrectomized rat. ROBERT A. BUSSABARGER and FREDERIC T. JUNG. From the Department of Physiology, Northwestern University Medical School, Chicago, Illinois. Work previously reported (Jung) led us to believe that the totally gastrectomized rat suffers from a nutritional deficiency. In the present work several procedures were tried upon a series of gastrectomized animals to determine the exact nature of this deficiency.

The procedures were:

1. Feeding a predigested food developed in this laboratory for jejunal feedings in dogs.
2. Boiling the "rough" constituents of the diet.
3. Addition to the diet of vitamin B in the form of brewer's yeast.
4. Addition to the diet of vitamin A and D in the form of cod liver oil.
5. Addition to the diet of liver extract no. 343.
6. Addition to the diet of ferric ammonium citrate and copper sulphate in the drinking water.
7. Subcutaneous injections of Phyone.
8. Subcutaneous injections of ferric ammonium citrate alone and together with liver extract no. 343.
9. Varying the daily diet.

This last procedure was apparently the best; but even a greatly varied diet ranging from fresh fruit to canned dog food and cracked corn did not cause any satisfactory gains in weight.

The failure of all the above procedures to affect materially the horizontal or downward trend of the growth curves leaves us unenlightened as to the exact nature of the deficiency.

Because of the high mortality a series of 225 rats were gastrectomized by the method, with a few modifications, reported by Jung and Jones. Of this number only 20 per cent survived longer than thirty days. The average length of life for the whole series was nineteen days. Causes of death in the order of their frequency were obstruction (approximately 70 per cent of deaths), local abscesses, hemorrhage, and cachexia.

No gross internal pathology could be detected in those rats which survived for 100 days or over. The animals were emaciated and did not keep themselves clean; their hair was coarse, scanty, and easily pulled out.

Nearly all of the obstructions were followed by pneumonia and death. The cause of obstruction varied with the survival time. Hair obstructions were the most frequent cause of early deaths, then food obstructions and stenosis became increasingly responsible for deaths. It was found that hair obstructions could be prevented by placing the rats in separate cages. By this procedure the survival period was increased to three times that of rats kept two in a cage.

The growth curves of the 45 animals which survived for over 30 days differed slightly from those previously reported. In the present series only a few animals regained their preoperative weights. There was a steady, postoperative decline in weight for about two weeks. Then, the weight curve leveled off or rose slightly to remain fairly constant for a month or so only to fall slowly till the animal died.

Blood findings (previously reported) in the gastrectomized rat as well as the effect of Fe injections were confirmed.

The distribution of sugar between corpuscles and plasma in hyper- and hypoglycemic conditions. DEA BAILEY CALVIN. Laboratory of Biochemistry, University of Missouri School of Medicine, Columbia, Missouri.

In a previous communication¹ it was shown that the distribution of sugar between corpuscles and plasma depended in large measure on the water content. If this was taken into consideration the ratio $\frac{\text{corpuscle sugar}}{\text{plasma sugar}}$ was practically unity. In the present investigations this work has been extended to cover abnormal sugar pictures. Preliminary results seem to indicate that insulin may play a part in controlling the permeability of the corpuscle to sugar. In its absence the corpuscle sugar content falls, with a corresponding fall in the ratio. Experiments are now in progress to clarify this point and to study the picture in phloridzin diabetes. *In vitro* experiments are also in progress.

Dogs have been used for the experimental work. Sugars were determined by the Shaffer-Somogyi² technique. Blood and plasma proteins were removed by zinc sulphate-sodium hydroxide and copper sulphate-sodium tungstate precipitants.

Serum phosphatase in cats with total bile stasis. A. CANTAROW, H. L. STEWART (by invitation) and S. G. McCOOL (by invitation). Laboratory of Biochemistry, Jefferson Hospital, and the Department of Pathology, Jefferson Medical College, Philadelphia. (Read by title.)

Previous morphological and functional studies indicate that the cat differs in certain essential respects from other species with regard to the effects of ligation of the common bile duct. It has been shown that obstructive and intrahepatic forms of jaundice in human beings and dogs are accompanied by a rather consistent and marked increase in serum phosphatase activity. Determinations of serum phosphatase and serum bilirubin were made in 30 adult cats at various stages of uncomplicated total bile stasis (confirmed at autopsy) of 2 to 29 days' duration. Phosphatase activity was determined by the method of Bodansky (J. Biol. Chem. 101: 93, 1933).

The control values ranged from 0.95 unit to 3.84 units. Of 45 determinations made during total stasis, 41 were within the limits of the control values (0.97-3.76 units; stasis 2-21 days' duration). Values above normal were obtained in only 4 instances: 11.03, 5.92, 5.8 and 4.6 units, with stasis of 16, 29, 21 and 8 days' duration, respectively. There was no consistent relationship between serum phosphatase activity and either the duration of stasis or the degree of bilirubinemia.

These observations indicate an additional point of difference between the cat and other species in regard to the effects of total bile stasis upon the organism. Studies are in progress to determine the relationship, if any, between the absence of increased phosphatase activity and the comparatively slight hemorrhagic tendency manifested by cats with total bile stasis.

¹ Calvin, D. B. This Journal 101: 16, 1932.

² Shaffer, P. A. and M. Somogyi. J. Biol. Chem. 100: 695, 1933.

The influence of temperature on the recovery heat-production of mammalian muscle. McKEEN CATTELL and EPHRAIM SHORR. Departments of Physiology and Medicine, Cornell University Medical College and the New York Hospital, New York City

Observations made on isolated strips of dog muscle in oxygen at 23.5°C. have shown that the ratio of the delayed heat to the initial heat varies between 2.5 and 3.5 for a short tetanus (Cattell and Shorr: This Journal, 101: 18, 1932). The initial process is probably not carried on with an efficiency greater than 35 per cent, so that such a ratio denotes a very low efficiency for the complete cycle, obviously much less than obtains in the intact animal where studies of the oxygen consumption in relation to work done show an efficiency of the order of 25 per cent.

Recent observations show that an important factor contributing to the low efficiency is the low temperature. In experiments made at 23.5°C. in serum the recovery heat averaged 2.8 times the initial heat in a 0.5 to 1 second tetanic contraction. At 37°C. similar experiments gave an average for the recovery heat equal to 1.2 times the initial heat. The latter value is somewhat lower than that found for a short tetanus in the frog's sartorius muscle at room temperature. A further point of interest is the great rapidity at which recovery takes place. At room temperature the delayed heat is all over in about 5 minutes and is thus much more rapid than occurs with frog muscle at the same temperature. At 37°C. the delayed heat comes on with great promptness, reaches its maximum rate within a few seconds after the end of the stimulus, and can only be separated from the initial heat by the method of differential analysis in small time units. Recovery is practically complete at the end of the first minute after stimulation.

Carbohydrate metabolism in the hypophysectomized dog. WILLIAM H. CHAMBERS, J. E. SWEET and J. P. CHANDLER (by invitation). Departments of Physiology and Surgical Research, Cornell University Medical College, New York City.

A method has been devised by Professor Sweet for approaching the gland through the temporal route with a minimum sized opening in the skull of about 10 to 15 mm. in diameter. The stalk is severed with a spherical instrument which removes the gland in toto and intact, thus allowing for an immediate determination of the success of the operation. Calorimeter, blood and urine studies have been made on 15 adult hypophysectomized dogs. The low basal blood sugar values reported by others have been confirmed. The average of 40 venous blood sugar determinations was 60 mgm. per cent in the operated animals as compared to 73 mgm. per cent for the same number of normal controls. This condition does not seem to be associated with an increased ability to oxidize carbohydrate. The basal non-protein respiratory quotients in 10 experiments were within the normal range and after the ingestion of 50 grams of glucose varied between 0.95 and 1.05. The low blood sugar could not be ascribed to an over production of insulin according to the insulin content of the pancreas. The assay was made by injecting the extract of pancreas into hypophysectomized dogs and comparing the fall in blood sugar with that obtained from standard insulin. The yield of insulin from the pancreas of hypophysectomized animals was not essentially different from the normal, ranging between 1.0 and 3.5 units per gram of wet tissue and averaging 2.6 units

in each group. Hypersensitivity to insulin has been confirmed and found to be about tenfold, i.e., 0.025 unit per kilo of body weight intravenously produced approximately the same decrease in blood sugar as 0.25 unit in the normal dog. The abnormal sensitivity to insulin was also evident when it was given simultaneously with glucose, but the respiration calorimeter experiments showed no increased oxidation of carbohydrate or transformation into fat. After fasting for 5 to 16 days the hypophysectomized animals were similar to the controls in exhibiting a decreased ability to oxidize ingested glucose. Two of the operated dogs after fasting for 24 and 30 days respectively gave no rise in respiratory quotient following the ingestion of 25 grams of glucose.

Plasma lipids in pancreatic atrophy following administration of betaine hydrochloride. B. B. CLARK (by invitation) and R. B. GIBSON. Laboratory of Pathological Chemistry, The State University of Iowa, Iowa City.

It has been pointed out by Best and co-workers that depancreatized dogs maintained on insulin develop fatty livers, but that if either raw pancreas, lecithin, choline or betaine is added to the diet these fatty livers can be prevented or cured. Chaikoff and Kaplan have recently shown that all of the blood lipid constituents of such dogs are decreased while the amount of fat in the liver is markedly increased.

We have studied the plasma lipids of a patient with an atrophic pancreas verified by exploration. She had a mild diabetes requiring about 15 units of insulin daily. Her stools were typical of pancreatic insufficiency. She has been observed on two periods during which she received betaine hydrochloride. When first seen in 1933 the total plasma lipid was somewhat elevated being 1002 mgm. per cent with an iodine number of 42; phospholipid phosphorus was 10.7 mgm. per cent. After supplementing her diet with brains and eggs for a few days the total lipid level diminished with an increase in the iodine number. After receiving betaine for 10 days, the total lipid was 622.5 mgm. with an iodine number of 64. Four months later the total fat was 710 mgm. with an iodine number of 69.

On discharge the patient was instructed to continue taking betaine which she did intermittently until a month before readmission January 1935. The total plasma lipid was 633.7 mgm. with an iodine number of 69. After 10 days on betaine the total lipid decreased to 563.7 mgm. and the iodine number increased to 77; the phospholipid phosphorus increased from 6.6 mgm. to 8.3 mgm.

Clinically the patient has shown marked improvement since the institution of betaine therapy. There has been no striking change in insulin requirement or in the character of the stools.

These data support the results of Best obtained on depancreatized dogs.

Afferent function in the group of nerve fibers of slowest conduction velocity.

DEAN CLARK (by invitation), J. HUGHES (by invitation) and H. S. GASSER. Department of Physiology, Cornell University Medical College, New York City.

Afferent function in the group of nerve fibers of slowest conduction velocity was investigated as follows: in cats under dial or amytal anesthesia stimulation of the saphenous nerve at a strength just sufficient to produce a maximal "C" elevation in the electroneurogram causes a more striking

effect upon the animal, as evidenced by respiratory changes, than does any stimulation below "C" strength. In deeply anesthetized animals, indeed, there may be no observable effect with a stimulus below "C" threshold but a marked effect when that threshold is reached. Furthermore, when all fibers except those contributing to the "C" elevation are blocked by compression of the nerve, an electrical or a mechanical stimulus of sufficient intensity still produces activity of the "C" fibers and striking changes in the cat's behavior.

Similar results are obtained when the block is produced through asphyxia, either by simple interruption of the circulation to the leg or by compression of the leg and included nerve with a sphygmomanometer cuff inflated above the systolic blood pressure level. With either method it was consistently observed that for 15 minutes no changes take place in the electroneurogram; that rapid disappearance of "A" and "B" activity occurs from 15 to 25 minutes after asphyxia is begun, and that the "C" fibers alone remain active after 30 to 45 minutes. These experiments furnish a striking parallel to those of Lewis, Pickering, and Rothschild, which were repeated here, using the sphygmomanometer cuff in man. In the human experiment no loss of sensation occurs for 15 minutes. From 15 to 25 minutes after inflation of the cuff there is rapid disappearance of touch, pressure, position, vibration, and cold sensations. After 30 to 45 minutes—at which time in the cat only the "C" fibers are active—delayed recognition of warmth and of a severe burning pain were the only responses obtainable by any sort or intensity of stimulus. From these observations it is concluded that nerve fibers of slowest conduction velocity do have an afferent function and are responsible for the conduction of impulses interpretable as pain and warmth.

The carotid sinus nerve in the dog. CHARLES F. CODE (introduced by F. C. Mann). Department of Physiology, University of Manitoba, and Division of Experimental Medicine, Mayo Foundation, Rochester, Minnesota.

I. *The innervation of the carotid sinus.* Three sources of nerve supply to the sinus have been found in twenty dissections of the carotid sinus region: 1, from the glossopharyngeal nerve making up the carotid sinus nerve; 2, from the superior cervical ganglion of the sympathetic, and 3, a minute twig which passes along the medial side of the internal carotid artery and may communicate with the nodose ganglion of the vagus.

II. *The functions of the nerves ending in the carotid sinus.* These have been investigated by means of 1, electrical stimulation; 2, varying the pressure within the isolated sinus in acute experiments and section of the different nerves to the sinus; 3, survival experiments in which the carotid sinus nerve was evulsed on one side. At a later date the sensitivity of the sinus to pressure changes was tested. The unoperated side was used as a control. Our results enable us to conclude that the cardiovascular components of the sinus reflex are conducted solely by the sinus nerve. Evulsion of this nerve in survival animals affords a method of removing the cardiovascular reflex effects of the carotid sinus without impairing the functions of the other nerves in the region or the blood supply to the head.

III. *The nervous pathway of the carotid sinus reflex.* The slowing of the heart which occurs on electrical stimulation of the carotid sinus nerve depends on both the vagal and sympathetic nerves to the heart, because

section of either of these pathways does not abolish the reflex. The vascular portion of the reflex is abolished by section of the spinal cord in the lower cervical region.

The mechanism involved in the production of exophthalmos in the dog by vago-sympathetic stimulation. CHARLES F. CODE (by invitation) and HIRAM E. ESSEX. Division of Experimental Medicine, The Mayo Clinic, Rochester, Minnesota. (Demonstration.)

It has long been known that stimulation of the cervical sympathetic nerves causes a marked exophthalmos but the mechanism by which the protrusion of the eye is accomplished is obscure. In a series of experiments on the dog we have demonstrated that the exophthalmos resulting from stimulation of the central end of the cut vago-sympathetic chain is due to a marked constriction of the fascia bulbi in a direction circular to the long axis of the orbit. The effect is more pronounced following section of the cervical cord. Isolated strips of the fascia bulbi in Ringer's solution respond to adrenalin by definite contraction. Histological preparations of the fascia reveal the presence of smooth muscle fibers.

*Electrical impedance of *Hipponoe* egg.* KENNETH S. COLE. Department of Physiology, Columbia University.

Alternating current resistance and capacity measurements have been made from $1.08 \cdot 10^3$ to $2.32 \cdot 10^6$ cycles per second on suspensions of unfertilized, fertilized and swollen unfertilized eggs of the Echinoderm *Hipponoe esculenta*.

The membrane of the unfertilized egg is practically nonconducting at low frequencies and shows a static capacity of $0.87 \mu\text{f}/\text{cm.}^2$ except perhaps at the highest frequencies. The equivalent specific resistance of the egg interior is 11 times that of sea water.

The membrane of the fertilized egg is practically nonconducting at low frequencies and shows a static capacity 2.5 times that of the unfertilized egg except at the higher frequencies where another reactive element produces a marked effect. The internal resistance is apparently higher than that of the unfertilized egg.

The static capacity per unit area of the membrane decreases as a linear function of the surface area when the eggs are swollen in dilute sea water. In 40 per cent sea water, the capacity falls to about 75 per cent of normal.

Although the membranes of *Arbacia* eggs and practically all tissue cells show a polarization impedance, the electrical characteristics of the unfertilized *Hipponoe* egg membrane are approximately those of the red blood cell and yeast cell membranes.

In view of the capacity increase on fertilization and decrease on swelling, an assumption of a dielectric constant and calculations of the membrane thickness are open to question.

The effect of ovarian hormones upon the B.M.R. of castrate women. MARY E. COLLETT, GRACE E. WERTENBERGER (by invitation), MARGARET SCHOTT (by invitation), FLORENCE LAWTON (by invitation), D. M. HARLOR (by invitation) and FAITH REED (by invitation). Department of Biology, Western Reserve University, Cleveland, Ohio.

Daily observations of the B.M.R., with and without medication, have been made over a period of weeks upon three castrate women. Subject A,

age 35, ovaries, tubes and uterus removed four years ago, B.M.R. minus 22 per cent; subject C, age 31, ovaries and tubes removed four years ago, B.M.R. minus 19 per cent; subject D, age 42, intensive radium treatment for fibroid eight years ago, B.M.R. minus 7 per cent.

Single doses of aqueous theelin (50 R.U.) or of oil theelin (300 R.U.) produce a transitory rise in B.M.R., which is larger and more lasting with the heavier dose. Repeated doses of these preparations at short intervals produce either a rise or a fall within 24 hours, followed by a sustained rise after medication has ceased. Menformon in aqueous and oil preparations acts like theelin.

In subject D, who differs from the others in age and in mode of castration, single doses of oil theelin generally depress the B.M.R. by 5 or 10 per cent at first, with a return to the normal level in about six days.

In subject A, a series of seven doses of corpus luteum extract (1 cc. on alternate days) produced a steady rise totalling 9 per cent above her usual level, which disappeared in about a week. In subjects C and D however, the effect was irregular, though a rise usually resulted.

Without daily observations many of these changes in B.M.R. would have been missed.

Theelin and corpus luteum checked the hot flashes of A and C in the second and third years after operation, but exaggerated them in the fourth year.

Fetal and early post-natal cardiac activity in the rat. E. L. COREY. Physiological Laboratory, University of Virginia.

The heart rate of 230 rat fetuses averaging 31 mm. in crown-rump length and weighing between 3.5 and 5.0 grams was graphically recorded under various experimental conditions. Decerebration (36 litters) or light ether anesthesia (20 litters) was employed as a means of immobilizing the mother. Asphyxia of the fetus or mother resulted in an irregularity (alternate slowing and acceleration, dropped beats, extra-systoles, etc.) of the fetal heart rate. Such irregularity in the fetal heart rate provided a convenient check on the normality of the preparations employed.

Lactic acid (0.1 per cent) dropped directly on the fetal heart or injected into the maternal circulation produced an irregularity similar to that seen in asphyxia; alkaline solutions administered similarly had no demonstrable effect on the fetal heart rate. The heart of the fetal rat was found to be highly sensitive to changes in the temperature of the surrounding medium.

The cardiac nerves were found to be functional in rat fetuses approaching term as evidenced by experiments involving vagal section and electrical stimulation of the fetal vagus, cervical sympathetic, and stellate ganglion. The results of atropine and pilocarpine administration were in agreement with the above observations. No reaction of the heart of the fetal rat to adrenalin or adrenin secretion could be definitely demonstrated.

Over 100 observations on cardiac action were also made on 50 newborn and young rats. Responses to atropine and pilocarpine were positive in practically all instances during the first week of post-natal life. In all cases, furthermore, the reactions to vagal and sympathetic excitation were likewise positive in five-day-old animals. It was noteworthy that definitely positive responses to adrenalin were first elicited in observations made on seven-day-old rats; thereafter they were found almost invariably

positive and similar to the adult type in tests made on animals between one and three weeks of age.

Growth and glycogen content of the fetal liver and placenta. E. L. COREY.
Physiological Laboratory, University of Virginia. (Read by title.)

The growth rate and glycogenic content of the fetal liver, as well as that of the maternal liver and the fetus as a whole, have been determined. The experiments involved over 300 rat fetuses comprising 48 litters. All observations were made on fetuses in the latter third of the gestation period.

The growth rate of the fetus as a whole exceeds that of the liver until a body weight of about 1.3 grams is attained; subsequently general bodily and hepatic growth progress at approximately the same rate. A slight but progressive hydration of the fetal liver was evident throughout the period of development studied. The placental water content remained, however, relatively constant. Placental growth was found to be rapid until the fetus attained a weight of about 3.0 grams, after which no notable change in the size of the placenta was detected.

At a fetal weight of approximately 0.3 gram the liver glycogen concentration in the fetus was found to first exceed that of the placenta. This stage probably represented the beginning of adult hepatic function in the rat. The placental glycogen concentration fell steadily from the earliest stages studied until term, although the maternal hepatic glycogen value showed wide variation throughout the series of animals employed. This finding was interpreted as indicative of a relative independence of the placenta to different maternal carbohydrate levels (i.e., liver glycogen).

Increased water exchange after Eck fistula in dogs. LATHAN A. CRANDALL,
JR. and GEORGE M. ROBERTS (by invitation). Department of Physiology
and Pharmacology, Northwestern University Medical School,
Chicago, Illinois.

Since Eck fistula animals are known to show a greater blood dilution and more prompt diuresis than normal dogs after the administration of water by mouth, it seemed possible that they might exhibit a tendency to drink at more frequent intervals with a resultant increase in voluntary intake and in urinary output.

In a study of daily intake and output in eight Eck fistula dogs before and after operation these expectations have been realized. Four of the animals have shown only a moderate increase in voluntary intake, which was of brief duration. In the other four dogs the intake increased to more than four times the preoperative level, and was maintained at a high level more than six weeks. Other dogs not studied preoperatively have shown abnormally large intake and output when measured four months after operation.

The excess of urinary output over water intake (exclusive of water in food) which is present in the normal animal tends to become less after the Eck fistula operation. Since there is no gain in weight these animals must also lose a larger amount of water by other pathways than is true of the normal dog. Studies of freezing point depression of the blood and of blood chlorides in the Eck fistula animals have given normal values. The specific gravity of the urine falls to low values during the period of increased water exchange following the operation.

Apparent specificity of the induced ovulation reaction in amphibia. CHARLES W. CREASER and AUBREY GORBMAN (introduced by T. L. Patterson). Zoological Laboratory, Wayne University, Detroit.

The lack of uniformity in the induced ovulation reaction in amphibia to pituitary and pituitary-like preparations has occasioned some question as to the constancy of the ovulation inducing or gonadotropic factors of pituitaries from the different members of the vertebrate sub-phylum.

The nature of this apparent specificity is being investigated using *Rana pipiens* as test animal, since this is a more convenient form and its sexual cycle is not as subject to complicating factors as that of the higher vertebrates. If it can be shown for this species that the apparent specificity is not due to such experimental conditions as physiological state of the specimen, size of dose, or condition of the preparation it may become possible to advance more strongly a hypothesis of hormone difference.

Negative results were obtained from *R. pipiens* with pregnant mare's serum; a pregnancy urine preparation, Antuitrin-S; a beef anterior lobe extract, Antuitrin; live implants and macerated injections of whole glands from the fishes *Perca flavescens*, *Cristovomer namaycush*, and *Stizostedion vitreum*. Size of dose has been checked by small to massive doses. Physiological state of the specimens has been checked by maintenance of good conditions and further checked by successful induction of extra-seasonal ovulation by means of homoplastic implants after failure with other preparations. Simultaneous frog gland implants and injections of extract gave ovulation, indicating that concomitantly introduced chemicals were not inhibiting possible action of the extract.

Effect upon cochlear function of intense tonal stimulation. ELMER CULLER and GLEN FINCH (introduced by F. R. Steggerda). University of Illinois.

1. Protracted stimulation at 1000 cycles is followed by a severe horizontal subsidence of auditory acuity throughout an extensive section of the audible range (at least 200 to 5000 cycles).

2. A given quantity of sound-energy extending over ten hours at 4000 cycles brought a loss of 55 db at 125 cycles, increasing from that point to a maximal loss of 104 db at 4000 cycles.

3. The same quantity of sound-energy at the rate of 250 cycles gave on the contrary no loss at any frequency from 125 to 8000 cycles.

4. Equal quantities of stimulation at 1000 cycles show the following losses at successive octaves from 125 to 8000 in decibels: with both tensors tympani intact, 6, 4, 6, 9, 8, 9, 7; with both tensors severed, 17, 13, 19, 17, 21, 21, 12. This indicates that the tensor tympani serves to protect the ear at high intensities.

5. Comparative effects of a given quantity of stimulation upon (a) acuity of hearing and (b) electric response of the cochlea will be presented.

6. When the tonal stimulus is interrupted about once per second, it induces far greater functional loss of hearing than does a continuous tone of the same amplitude. A well known engineering phenomenon (repeated stresses are more destructive than a dead load of the same magnitude) is thus seen to hold for the cochlea.

Studies on black widow spider venom. FRED E. D'AMOUR. Research Laboratories, University of Denver.
During the past year this spider has shown an alarming increase in

numbers and extent of range. Although timid by nature, their increased number has resulted in an increased incidence of arachnidism. The bite is dangerous and occasionally fatal. In the study here reported, some 1000 spiders and 300 rats were used. Colored slides showing different stages in the life cycle will be shown.

A technic for dissecting the glands was developed to overcome the lack of consistency found in actual biting experiments and to supply a pure solution of venom.

Toxicity studies were made and a toxicity curve established, the average lethal dose for young rats was found to be 0.032 mgm. of dried venom.

Chemical studies indicate that the venom is a protein, probably an albumen. It is easily denatured by mild chemical reagents.

A potent antiserum was prepared, of which $\frac{1}{10}$ cc. completely protects rats against at least 8 A.L.D. when given immediately, and of which 1 cc. gives prompt recovery when injected $3\frac{1}{2}$ hours after administration of 8 A.L.D. of venom.

No antidote was discovered among the various agents tested.

Histologic study of the tissues of rats poisoned both acutely and chronically revealed no pathology.

Studies on urinary hebin. M. C. D'AMOUR and F. E. D'AMOUR. Research Laboratories, University of Denver.

Urinary hebin was prepared by the method of Wallen-Lawrence and van Dyke from a composite lot of pregnancy urine made up from 108 samples taken from all periods of pregnancy. Fifty-seven hundred cubic centimeters of the urine yielded 9.85 grams of preparation. This was injected in different dosages into a series of groups of 20 each of immature male and female rats. The injections were begun on the 21st day of life and continued for three days. The animals were sacrificed 100 hours after the first injection. The average results were as follows: Males, weight of seminal vesicles on total dosages of 0, 1, 1.5, 2, 4, 8 and 16 mgm. of preparation, were 7.6, 12.6, 14.7, 21.8, 23.2, 25.5 and 27.8 mgm. respectively; females, weight of ovaries on total dosages of 0, 2, 4, 8, 12, 16, 24, and 48 mgm. of preparation, were 10.9, 12.0, 13.9, 20.1, 23.6, 26.5, 31.2, and 35.5 mgm. respectively.

Curves plotted from these figures show, in the case of the males, a sharp rise with a sudden flattening out, while in the case of the females, the rise is very gradual and there is no sharp break. This might indicate that the male would be better for assay purposes and that a dosage resulting in seminal vesicles weighing 15 mgm. would be most satisfactory. When weights of seminal vesicles or of ovaries were chosen which fell on the steepest part of the curve and the per cent of animals in each group having such a weight or over was plotted against the dosage, sharp S shaped curves resulted. Thus, when 20 mgm. was arbitrarily chosen as the minimum weight of ovaries, 5, 10, 60, 85, and 100 per cent were positive on dosages of 2, 4, 8, 12 and 16 mgm. respectively. If 15 mgm. was arbitrarily chosen as the minimum weight of seminal vesicles, 20, 50, 80 and 100 per cent were positive on dosages of 1, 1.5, 2, and 4 mgm. respectively. From such characteristic curves, the dosage affecting 50 per cent of the animals can easily be read, and such an amount could serve as a satisfactory unit. Similar curves resulted when other weights were chosen which were close

to those used above. The curves were not satisfactory when the weights chosen were either very large or very small.

The mechanism of auditory masking. H. DAVIS and A. J. DERBYSHIRE (by invitation). Department of Physiology, Harvard Medical School.

Auditory masking is the diminution of audibility of one sound caused by the presence of a second sound. Study of the electrical responses of the cochlea and of the auditory nerve of the cat shows that this phenomenon depends primarily on the refractory period of the nerve fibers.

The cochlear response, which represents the activity of the sensory cells, shows no interference between simultaneous tones. There is simple addition of the electrical waves, subject only to the non-linear distortions of the ear.

The volley of action potentials which results from stimulation by a single click may be wholly or partly masked by a tone of 500 c.p.s. (both 20 db above threshold) or it may wholly or partly mask the impulses corresponding to one cycle of the tone, depending on the phase of the tone in which the click falls. The clicks and the tone compete for the same fibers and either one may exclude the other, for either response leaves the auditory pathway unresponsive to the other stimulus for roughly 1.0 ms.

Closer analysis is complicated by the multiple character of the response to the clicks. Two or three fairly well-defined groups of action potentials, differing in threshold and latency, usually appear. Each is masked independently of the others at different phase relations of the stimulating click with the masking tone.

Masking the response to clicks with tones of higher frequency (1000 to 4000) indicates that the earlier groups of action potentials are preferentially masked by the high tones and the later groups by the middle-range tones.

Masking is the only limitation of auditory function that as yet appears to be dependent on the all-or-none character of nerve conduction.

The human electroencephalogram. H. DAVIS, F. A. GIBBS (by invitation) and E. L. GARCEAU (by invitation). Department of Physiology, Harvard Medical School. (Demonstration.)

Trauma in strychnine poisoning. W. T. DAWSON, D. DUNCAN (by invitation), E. D. HOLLAR (by invitation) and C. H. TAFT, JR. (by invitation). Departments of Pharmacology and Anatomy, University of Texas, School of Medicine. (Read by title.)

Even the best works of reference tell little or nothing of a risk of trauma in strychnine poisoning. We were therefore surprised that over a fifth of the rabbits in a series injected subcutaneously with 1.0 mgm. per kgm. strychnine sulphate, thrown into tetanic state, and surviving through the intravenous injection of amylen hydrate or barbiturates, e.g., evipal, oral, phanodorn, sandoptal, showed impairment of movement in the hind limbs. Eight such rabbits were examined carefully by one of us (D.D.). Two recovered completely and showed at necropsy no gross or microscopic abnormality. Two died, 4 were sacrificed. The gross lesions found, with the number (if more than one) of animals showing each lesion, included: Hemorrhages into muscles, 3; retroperitoneal tissues of the pelvis, 2; a lumbar nerve trunk, and subarachnoid space, 2; rupture of pectenius; rupture of last lumbar nerve; luxation of sacro-iliac, hip, and knee-joints;

displacement of sacrum forward and medial to the lumbar vertebrae. Microscopically: marked wallerian degeneration in sciatic nerve, 2; marked chromatolysis of spinal ganglion cells and spinal motor cells, same 2; fragmentation of myelin, 2; inflammatory reaction about stump of ruptured nerve trunk proved premortem origin of injury: sections of hemorrhagic areas indicated that they were not the result of necropsy handling. Conclusions: In work on strychnine antidotes the morphinized, decapitate, or decerebrate preparation is indicated. A practical strychnine antidote should be a moderately persistent depressant like sodium amytal which, given by intravenous injection, terminates the convulsions promptly, and in adequate dose renders their recurrence unlikely (Kempf, McCallum, and Zerfas).

The probable mechanism for stimulation of the auditory nerve by the organ of Corti. A. J. DERBYSHIRE (by invitation) and H. DAVIS. Department of Physiology, Harvard Medical School.

We have studied the electric phenomena at the round window and the action potentials of the auditory nerve of the cat. The ear was stimulated by a series of clicks at 60 per second. Each click was a rapidly decrementing high-frequency train of sound waves.

At the round window, the latency of the action potentials from the first negative peak of the cochlear response was 0.8 ms. at threshold, 0.5 to 0.6 ms. at 30 db above threshold, and did not diminish with further increase in intensity.

The difference in latency between the first action potentials at the round window and the earliest response of the auditory nerve was never less than 0.09 ms. This yields a reasonable conduction rate of 44 meters per second and leads us to believe that no earlier action potentials escaped detection at the round window. The minimal latency of 0.5 to 0.6 ms. cannot be explained by a conduction time in the non-medullated terminations of the auditory fibers lying on the basilar membrane, because the shortest non-medullated sections are only about 30μ long.

We have previously determined the functional refractory period of the auditory fibers as 1.0 ms. This is evidence that these fibers are "quick" throughout. Their minimal utilization time could hardly be over 0.1 ms. Therefore the observed latency of 0.5 ms. cannot reasonably be explained as utilization time of stimulation by the cochlear response.

The polarity of the cochlear response adds a difficulty for any simple theory of direct electric stimulation, because the base of the hair-cells becomes electrically more positive at the time of stimulation.

We believe it probable, therefore, that some chemical mediator, liberated by the sensory cell, stimulates the nerve endings.

Factors governing the distribution of barbiturates. JAMES M. DILLE (introduced by Theodore Koppanyi). Department of Pharmacology of Georgetown University School of Medicine, Washington D. C.

The rate of disappearance of barbiturates from the blood, and the rate of their accumulation in the various organs was quantitatively studied in dogs and rabbits. After intravenous administration of barbiturates blood samples were taken at frequent intervals. These experiments showed that the rate of disappearance of the drug from the blood varies inversely with

the size of the dose. Conversely the organs take up barbital relatively faster after small than after large doses.

The rapidity of the taking up of barbital by an organ is different for different structures. Two hundred and fifty milligrams of sodium barbital by saphenous vein gave the following: after one minute, liver 0.50 mgm. per gram, muscle negative; after five minutes, liver 0.60, and muscle 0.10 mgm. per gram; after ten minutes liver 0.40, and muscle 0.24 mgm. per gram; after twenty minutes liver 0.40, and muscle 0.32 mgm. per gram.

Barbiturates were administered by various routes in order that the drug be presented first to a given organ before it reached and became diluted by the general circulation. If sodium barbital in aqueous solution is injected into the internal carotid artery the barbital content of the brain will be the same as after the intravenous administration of the same amounts. However, if barbital is given dissolved in 50 per cent methyl alcohol and injected into the carotid artery, the brain will take up far larger amounts of this drug. For example the cerebrum contains 0.269 mgm. of barbital per gram and the liver 0.171 mgm. per gram after the administration of 300 mgm. of barbital per kilogram in 50 per cent methyl alcohol into the carotid artery, whereas if the same dose is injected in aqueous solution by the same route the brain contained only 0.06 and the liver 0.1 mgm. per gram.

The nembutal concentration of the brain is high no matter by what route or method the drug is injected. If, however, sodium barbital in aqueous or alcoholic solutions is injected into the portal vein the liver is capable of retaining large amounts of barbital for a relatively long period.

On the renal excretion of urea. R. DOMINGUEZ (introduced by A. J. Carlson). Saint Luke's Hospital, Cleveland.

It is known that the rate of excretion per unit plasma concentration (clearance) has a mean value independent of diuresis for moderate and large diuresis, and that for small diuresis the clearance diminishes as the urine flow diminishes. This behavior may be explained by considering the rate of excretion y as the resultant of two operations: First, the rate s at which urea is poured into the lumen of the tubules, and second, the rate r at which urea is absorbed from the lumen of the tubules. With the help of two assumptions concerning the nature of the two rates, s and r , namely, 1, that at any diuresis the rate s is proportional to the plasma concentration, and 2, that the rate at which urea is absorbed varies with diuresis in such a way that the change in the rate of absorption, r , with respect to diuresis, v , is proportional to the rate of absorption r , the following equation is found for the clearance C ,

$$C = A (1 - e^{-kv}),$$

where A is the asymptotic mean value of the clearance as diuresis increases. The dimensions of A and k are those of flow and reciprocal of flow, respectively. Therefore, the dimensions of the clearance at any diuresis are those of flow, as required by definition. The notion of an augmentation limit disappears, but the term can be used to designate a range of asymptotic approach to A .

The equation was fitted to the mean clearance per interval of diuresis in 432 observations on 49 dogs taken from independent sources. The deviations of the points from the curve are smaller than those from the

curve of Austin, Stillman and Van Slyke, $C = b \sqrt{v}$. The variance in the first case is 0.9889, in the second 2,5693. The values of the constants are $A = 34.95$ (mixed diet), $k = 7.76$.

The equation leads to a limiting concentration ratio (Ak) as diuresis approaches zero. If the independent assumption is introduced, that (Ak) remains constant with dietetic changes in A , the equation explains the shift in the augmentation limit with change in A , as observed by Jolliffe and Smith (1931).

Influence of the female white rat on bodily activity of the male. E. P. DURRANT.

Department of Physiology, Ohio State University.

White rats in self-recording activity cages were arranged in isolated groups of three each, the female between two males with the three cages in line and the convex rotating surfaces of the rotating cages within an inch of each other. After the occurrence of six or eight estrous cycles, the number varying somewhat in the different females, individual activity graphs of the usual type were constructed. Study of these graphs showed that whereas a number of males placed in a separate room and in close proximity do not show such wide variations in activity from day to day, nor the characteristic four to five day high peaks as seen in females under like conditions, in this experiment the males took on a rhythmic variation agreeing in 85 per cent of instances with that of the associated female.

Is the work of the kidney, due to the excretion of urea, a factor in specific dynamic action? A. G. EATON, SHIRLEY CORDILL (by invitation) and J. L. GOUAUX (by invitation). Department of Physiology, Louisiana State University Medical Center.

Experiments were performed on four healthy human subjects, the three authors and a departmental technician, all of whom were well trained and whose basal metabolism had been determined frequently before the experiments began. One basal determination was made each morning followed by the ingestion of 30 grams of urea dissolved in water at body temperature, except in a few instances when 10 to 20 grams of urea were given. Successive hourly determinations of basal metabolism and nitrogen excretion were made for four hours. Basal metabolism was determined by collection of expired air in a Tissot spirometer, analyses were made on an extremely accurate Haldane apparatus and nitrogen was estimated by the usual maero-Kjeldahl method. Control days without urea were interposed. Nineteen experiments were performed.

Although the nitrogen excretion often increased 1 to 2 grams per hour over the basal output and the specific gravity of the urine increased greatly, there was no consistent rise in the basal metabolic rate. Minor fluctuations occurred in both directions but a slight fall was more frequently observed than an increase. The results confirm the experiments of Lusk and of Grafe but are opposed to those of Borsook and Winegarden. The work of the kidney in the excretion of urea is not a factor in the specific dynamic action of proteins.

The action of pressure on the tension response of smooth muscle. DAYTON J. EDWARDS. Department of Physiology, Cornell University Medical College, New York City. (Read by title.)

A strip of muscle about 1 cm. long and 3 to 4 mm. wide obtained from

the pyloric end of the stomach of the "painted" terrapin was used in these experiments. The method of mounting the muscle and of applying pressure was similar to that employed in this laboratory in the work with cardiac and skeletal muscle. A faradic stimulation of the muscle of 10 seconds' duration with measured rest periods of 4 minutes between the responses gave consistent results.

The records for 17 experiments, with pressures ranging from 200 to 1500 pounds per square inch and at a temperature of about 22°C., show a reduction of 54 per cent in the average amplitude of the response under pressure as compared with the average value for the control series. The action of pressure in reducing the size of the response occurs immediately, as timing its onset to coincide with the latent period, or with the beginning of contraction never failed to depress the ensuing response. Complete reversibility of the effect was evident in all instances on release of the pressure (i.e., with compression periods not exceeding 15 min.) and frequently the first response following the release was larger than the control. In a series of three or more contractions taken while the pressure was maintained the height became progressively larger indicating some sort of an adaptation.

A comparison of the tension-time of the muscle response under pressure with the control shows that the rate of developing tension is only about one-half as fast when pressure is acting upon it. However, if the compressed muscle is given a stronger stimulus the difference in rate and magnitude of the response becomes less. A certain amount of proportionality is indicated between the reduction in the contraction amplitude and the amount of pressure acting on the muscle but this relationship holds only for low pressures. At pressures of 5000 pounds on the muscle a contracture develops which may exhibit a total tension in excess of that obtainable with a maximal faradic stimulation.

Observations on life-maintaining and gonadotropic extracts of the adrenal glands. M. EHRENSTEIN (by invitation) and S. W. BRITTON. Physiological Laboratory, University of Virginia Medical School.

A number of modifications of an earlier method of preparing cortico-adrenal extracts, employed for some time past in this Laboratory, have recently been made. Higher yields of the hormone and more potent preparations containing the life-maintaining factor are now uniformly obtained. It has also been found possible to prepare by various methods extracts which produce gonadotropic effects, similar to those obtained by Evans and others from anterior pituitary lobe preparations.

Erythrocytic fragility and splenic derivatives. II. M. M. ELLIS, H. L. MOTLEY (by invitation) and M. D. ELLIS (by invitation). Department of Physiology, School of Medicine, University of Missouri.

The actions of several splenic derivatives on the fragility of erythrocytes from various laboratory animals as previously described by us (*J. Pharmacol. Exp. Therap.*, in press), have been extended to erythrocytes of the monkey, *Macacus rhesus*, and of man. Definitely hemolytic and anti-hemolytic fractions from splenic extracts and from commercial preparations of spleen have been obtained as in previous work. The reactions of the human and monkey erythrocytes resemble those of erythrocytes of dog and cat more closely than any of the other experimental animals previously tested. The

same types of protective and hemolytic reactions were also obtained with splenic preparations acting on erythrocytes from human patients suffering with purpura hemorrhagica, obstructive jaundice, pleursy, streptococcus sore throat and pulmonary tuberculosis, and on erythrocytes of splenectomized dogs and monkeys.

X-ray treatment of short duration in rats. F. E. EMERY and G. W. THORN
(by invitation). University of Buffalo.

The heads of rats were exposed to x-ray treatment for a period of ten to twelve minutes at one time and were kept under observation without further treatment for several weeks. In the albino females with normal estrous cycles before treatment no effects were observed in the frequency or character of the estrous cycles after treatment. In the hairless females where the estrous cycles are irregular and frequently of several days or weeks in length the same x-ray exposure brought about normal cycles in most cases. In this hairless colony there is reason to believe that the pituitary gland is not functioning in a normal manner but does so after the x-ray treatment. The beneficial effects last for several weeks.

The blood flow in the left coronary artery of the intact dog. H. E. ESSEX, J. F. HERRICK, E. J. BALDES and F. C. MANN. Division of Experimental Medicine, Mayo Clinic, Rochester, Minnesota.

A vast amount of data has been amassed on the bloodflow in the coronary arteries of the dog but heretofore all such information has been obtained on animals under deep anesthesia. By means of the thermo-stromuhrl method of Rein and the use of a specially designed unit observations have been made repeatedly on a series of animals, some of which have remained in good condition for as long as five and six days during which time they walked about the laboratory, took food voluntarily and behaved in a normal manner. The unit is applied to the vessel under ether anesthesia and with sterile technic. The heart is approached by a lateral incision through the fourth intercostal space. The pericardium is incised sufficiently to expose the proximal portion of the left coronary and its branches. After the vessel has been dissected free the unit is placed on the anterior descending or the circumflex branch of the left coronary. The chest is closed as in other operations within the thoracic cavity. Observations have been made on the effect on the coronary bloodflow of 1, digestion of a meal taken voluntarily; 2, exercise; 3, glucose given intravenously, and 4, drugs such as adrenalin and ephedrine. The bloodflow in the coronary arteries is decidedly increased during the digestion of food. As might be expected the bloodflow was increased by exercise. Glucose given intravenously did not produce a prolonged increase. The drugs mentioned caused an increase in the coronary bloodflow amounting to as much as 300 per cent.

The adrenal cortex and endogenous carbohydrate formation. GERALD EVANS
(introduced by D. W. Bronk). George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia.

In a previous communication it was shown 1, that when fasted rats were kept for 24 hours at $\frac{1}{2}$ atmosphere, carbohydrate newly formed from protein or fat and equivalent to 35 per cent of preexisting stores was laid

down, chiefly in the liver, and 2, that this phenomenon did not occur in adrenalectomized animals.

It is now shown that a 32 per cent increase in urinary nitrogen accompanies this new formation of carbohydrate. Fasting adrenalectomized rats excrete 20 per cent less nitrogen than intact controls and do not show any increase at $\frac{1}{2}$ atmosphere.

Frequent small doses of epinephrine for 24 hours lower the total glycogen of fasting rats; but nine animals from which the adrenal medullae were successfully removed did, when placed for 24 hours at $\frac{1}{2}$ atmosphere, have an average of 1 per cent liver glycogen and a value for gastrocnemius glycogen well above the normal fasting level.

Two hypophysectomized rats kept at $\frac{1}{2}$ atmosphere for 24 hours did not lay down liver glycogen.

Urinary ketones were heaviest in those animals in which the conversion occurred.

Studies on the electrical response of the cochlea. J. A. E. EYSTER, T. H. BAST (by invitation) and M. R. KRASNO (by invitation). University of Wisconsin Medical School.

These experiments are concerned with the origin and distribution of the electrical currents of the internal ear resulting from sound stimulation. The cochlea of guinea pigs were exposed and leads made from either the apex or base of the cochlea and an indifferent region in the neck muscles to an amplifier, attenuator, rectifier and swinging coil galvanometer. Tones of various frequencies from an audio oscillator and loud speaker were applied to the ear. The input intensity was controlled by an alternating current voltmeter and the voltage output from the cochlea recorded from the galvanometer. Comparisons of the output were made from apical and basal leads and after injury to or removal of portions of the cochlea. The experimental injury was determined by histological study. The responses to different frequencies on comparison of apical and basal leads indicate a rather high degree of frequency localization in the cochlea. The response to frequencies below 500 cycles is greater from apical leads, while above this frequency a larger response is obtained from basal leads. The localization of response to low frequencies in the apical portions of the cochlea is also indicated by their great reduction or abolition on removal of the apical or two upper coils of the cochlea.

Response of considerable magnitude may still be obtained at frequencies above 200 cycles after removal of all turns except the basal turn. Complete destruction or removal of the cochlea abolishes the response to all frequencies. Puncturing the canals of the cochlea, with drainage or withdrawal of fluid contents results in varying degrees of reduction of response. Disturbance of pressure relations appears to be the main factor concerned. The usual result is a sharp drop in response on opening the cochlea, followed by a gradual recovery as the disturbed pressure relations are readjusted. The integrity of Reissner's membrane and the presence of a cochlear duct distinct from the scala vestibuli is not essential. Approximately normal response may be obtained in the presence of advanced atrophy of the organs of Corti. In certain cases however of more or less localized atrophy, there is a reduction in response to the frequencies that would be expected and it may be that the atrophy or some associated pathological change is responsible.

Muscle force at different velocities of shortening. W. O. FENN. Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, New York.

Measurements were made by an optical isotonic lever of the speed of shortening of frog sartorius muscles under different loads. Velocities were measured at the same muscle length for all loads and at a point on the curve where acceleration was absent so that the force on the muscle is equal to the load. The force decreases with increasing velocity or the velocity decreases with increasing load. The force-velocity curve is not linear as would be expected from Hill's equation which attributes the decrease of force to the viscous resistance. Instead it is an exponential curve, following the equation $W + kv = W_0 e^{-av}$. Here kv is the viscous resistance, the total force exerted by the muscle being W (the load) plus kv . This total force decreases exponentially as the velocity increases. This curve is not consistent with the two-component muscle model consisting of one damped spring and one undamped spring in series which has been so successful in explaining the mechanical behavior of muscles in quick release experiments. In these experiments the muscle tension is constant during the shortening so that the undamped muscle component cannot participate. The damped component should follow Hill's equation but gives an exponential relation instead. It is concluded that the rate of shortening is conditioned by the rate of the chemical reactions which liberate the excess energy necessary for shortening rather than by the behavior of the muscle as a simple viscous-elastic system.

Further studies on the influence of the adrenal glands on experimental diabetes.

H. WARD FERRILL (by invitation), J. M. ROGOFF¹ and B. O. BARNES. Department of Physiology, University of Chicago.

In previous work we found some evidence indicating that removal of one adrenal or the removal of one and suppression of the epinephrine secretion of the other, may modify experimental diabetes in the dog. Extending these observations we found that if the animal is kept in good condition by a high carbohydrate diet and sufficient insulin to control the glycosuria, the apparent influence of the adrenals was more evident. The present report is based upon studies on 15 dogs. In some cases the pancreas was removed first and, after the animal's insulin requirement was determined, the adrenal operations were performed in two stages. In other cases, the adrenal operations were done first and several months later the pancreas was removed. The general results were similar in both series. For a period after either operation, the animal may require considerable insulin, but as its physical condition improves or other factors, as yet undetermined, participate, the insulin can be reduced gradually until a new level is reached. This does not appear to be associated with declining physical condition of the animals since it has occurred in cases when the animal was gaining in weight.

In some of the animals, after determining the insulin requirements we made determinations of residual epinephrine output, using the method of Stewart and Rogoff. In some animals requiring less insulin, a marked reduction in the epinephrine output was found. Two animals, requiring very little insulin, were injected with epinephrine constantly for 48 and 96 hours, respectively. The rate of injection was not more than 0.0002

¹ Aided by a grant from the Commodore Beaumont Foundation.

mgm. per kilo body weight per minute. The insulin requirement rose to the range for pancreatectomized dogs in which the adrenals are not subjected to operation. Following the cessation of epinephrine injection, the insulin requirement promptly returned to the previous level.

The estimation of pericardial capacity in living dogs. M. H. FINEBERG (introduced by C. J. Wiggers). Department of Physiology, Western Reserve University Medical School, Cleveland, O.

The question has repeatedly been raised, how soon the inelastic pericardium limits dilatation of the ventricle and hence its compensatory response to increasing dilatation. The subject has been studied from a number of angles, but the conclusions have not been entirely concordant. (For reviews cf. Wilson and Meek, This Journal 82: 34, 1927; Wiggers, Ergeb. der Physiol. 29: 250, 1929.)

The effective capacity can be measured directly in living animals by determining the maximum volume of warm saline that can be introduced into the closed pericardial sac without producing an impairment of cardiac filling as evidenced by the first definite rise of venous pressure. Starling (Lancet, 1: 652, 1897) reported one such observation showing that an increase of 40 cc. produces no effect on venous pressure. Additional measurements were therefore made on dogs with normal venous pressures shortly after complete barbital anesthesia and opening of the chest. The pericardio-diaphragmatic attachments were left intact and during the tests artificial respiration was regulated so that the animals just failed to breathe naturally. In twenty dogs ranging in weight from 7.5 to 16.5 kilos from 21 to 79 cc. of warm saline could be introduced without any impediment to ventricular filling. A tabular study of results shows at a glance that a general relationship exists between pericardial capacity, heart weight and body weight. Considerable variation occurs, however, in different animals, as shown by the fact that the ratios $\frac{\text{heart weight}}{\text{pericardial capacity}}$ varied

between 1.4:1 and 3.9:1, but the majority ranged around a 3:1 ratio.

If these ratios are applicable to normal human hearts weighing 250 to 300 grams then a human pericardial capacity of about 80 to 100 cc. can be inferred.

Since the values obtained experimentally in dogs and those inferred for man at least equal the stroke volumes for one ventricle, the conclusion is justified that the pericardial sac offers an ample margin by which the normal heart may dilate.

Volume changes of heart and of skeletal muscle during and after activity. ERNST FISCHER (introduced by W. O. Fenn). University of Rochester.

Meyerhof's method of measuring the volume change was applied to strips of turtle hearts. A temporary decrease in volume occurs nearly parallel to the mechanogram, but always preceding the latter for about one-fifteenth of its duration. At the end of the beat the volume is nearly the same as before the beat. After a single beat a volume change of small velocity occurs for a period several times longer than the duration of a beat. The direction of this volume change can vary within a series of consecutive beats; most times during the first intervals an increase occurs. Considering the smaller power a heart muscle can develop compared with skeletal muscle, approximately the same magnitude of volume change can be observed in both. Adopting Meyerhof's explanation for the volume

changes the energy for the heart beat is set free by the splitting of phosphagen or adenylyl pyrophosphate and, before relaxation is completed, practically full resynthesis has occurred. The energy for the recovery process originates only partly from glycolysis.

In experiments with frog gastrocnemii Meyerhof's results were confirmed with the important exception that under high initial tension during the contraction generally instead of a decrease in volume an increase could be observed. Using sartorii or semimembranosi such a reverse effect occurred already under a relatively smaller initial tension. Thus the physical conditions influence the volume change during muscle activity. According to all we know the average fibre length and tension during contraction govern the energy output quantitatively (Fenn effect). Before the conclusion can be drawn that there exists also a qualitative Fenn effect, the possibility must be excluded that the volume changes during contraction are at least partly caused by physical changes inside the muscle fibres.

The effect of bulbocapnine upon the venous blood pressure. A. FOGELBERG (by invitation) and C. E. LESE. Department of Physiology, The George Washington University Medical School.

It is believed that peripheral vascular changes are directly concerned with the development of fatigue in continuously stimulated nerve muscle preparations *in situ*.

Venous pressure measurements may aid in the determination of the character of the peripheral vascular picture.

Decerebrate cats and etherized dogs are being used. Venous pressure is recorded directly and continuously before and during bulbocapnine intoxication. These experiments are a part of a general set up in which the fatigue is measured in the gastrocnemius and soleus muscles.

The results show a slight but increased venous pressure after bulbocapnine administration. The drug has been shown to produce a fall in arterial pressure and some cardiac depression. It is suggested that the chief factor responsible for the venous pressure change is peripheral dilatation, but this circulatory change is of a nature which is not physiologically beneficial to the activity of the muscles studied.

The effects of ether, nembutal and avertin on the potential patterns of the motor cortex. A. FORBES, A. J. DERBYSHIRE (by invitation), B. REMPEL (by invitation) and E. LAMBERT (by invitation). Department of Physiology, Harvard Medical School.

The cortical potentials are recorded by means of silver-silver-chloride electrodes on corresponding points of the cat's motor cortices connected to a direct-coupled amplifier and string galvanometer. The cortex under the grounded electrode is cauterized.

Under moderate ether anesthesia, the cortex produces waves of 30 to 60 per second and about 25 microvolts and occasional waves of 1 to 3 per second and about 50 microvolts. Under moderate nembutal, the dominant picture is a series of excursions of 200 μ v or more, at 5 to 20 per second. Frequent bursts of greater activity also appear. Under moderate avertin, monophasic excursions (uninjured cortex usually negative) of over 200 μ v appear at 5 to 10 per second. These excursions appear in groups, separated by intervals of little activity.

Increased depth of ether anesthesia causes depression of fast waves and some decrease in the frequency of the slower waves. Deep nembutal

does not depress the amplitude at first but produces grouping of the excursions; deeper narcosis produces loss of amplitude without change of frequency. Deep avertin causes the same sequence as nembutal.

Maximal repetitive stimulation of the sciatic nerve contralateral to the intact cortex produces an increased amplitude of the type of waves already present. Under moderate ether, it also causes a shift of the baseline, indicating negativity of the uninjured cortex. Stimulation under nembutal produces an increase in amplitude of the 5-to-20-per-second excursions already present. Under avertin, a sharp negative spike appears at roughly 30 milliseconds after the onset of stimulation. If slow waves are present under ether or nembutal, stimulation of sciatic may abolish them.

It is possible to shift reversibly from any one of these patterns to any other, by an appropriate change of anesthetic. The pattern during sciatic stimulation follows these same changes.

Because the changes of pattern induced by these anesthetics follow parallel courses in spontaneous activity and in that evoked by stimulation, we conclude that they act not merely by blocking afferent impulses, but by modifying directly the activity of the cortex.

Unimolecular films of nerve proteins. LYMAN FOURT (by invitation), RICHARD S. BEAR (by invitation) and FRANCIS O. SCHMITT. Department of Zoology, Washington University, St. Louis. (Read by title.)

With an Adam trough for obtaining force-area curves and a vibrating condenser with tuned amplifier to measure surface potentials, the mechanical and electrical properties of unimolecular films of nerve proteins were investigated. Four proteins were extracted from lobster nerves. These consist of one nucleoprotein extracted at pH 6-12, another extracted at pH 13-14 (neurostromin), an acid-insoluble metaprotein, and a thermostable protein insoluble in solutions of pH 11-13. The last two are obtained from the first nucleoprotein.

The force-area curves of the two nucleoproteins were found to be similar. The curves show the typical sol-gel transition and no other definite point of curvature. In this respect these proteins resemble fibrinogen and differ from myosin, the latter showing an inflection in the rising portion of the curve. The acid insoluble metaprotein gives similar curves except that the metastable curve diverges from the equilibrium curve markedly. The alkali-insoluble protein does not spread at pH 3 or 7.9. Neurostromin spreads to a fluid film whereas the other proteins form elastic patches.

At pressures less than that required to produce the sol-gel transition the potentials fluctuate. At higher pressures the values become consistent and rise sharply and linearly. At pressures of about 16 dynes neurostromin films at pH 3 level off to a constant potential.

Effect of cortico-adrenal extract on hemolysin formation in normal adult animals.¹ C. A. FOX (by invitation) and R. W. WHITEHEAD. Department of Physiology and Pharmacology, University of Colorado School of Medicine, Denver, Colorado.

The resistance of animals to toxins, drugs and infectious processes is

¹ Aided by a grant from the Committee on Therapeutic Research of the American Medical Association.

markedly lowered by adrenalectomy. Likewise, the ability to produce immune bodies to antigenic substances is greatly diminished in adrenalectomized animals. Immunization of animals to toxins and other antigenic substances produces hyperplasia of the adrenal cortex with a concomitant increase in the cortical lipoids. The present investigation deals with the administration of cortico-adrenal extract to normal adult rabbits and rats in the process of immunization against sheep red blood cells.

In the first groups of experiments, 48 normal male rats, 2½ to 3 months old, were used. Of these, 16 received 1 cc. of cortico-adrenal extract two days prior and three days subsequent to the intraperitoneal injection of 0.5 cc. of a 10 per cent suspension of sheep cells; 16 rats were administered cortico-adrenal extract inactivated by heat, in order to rule out a non-specific factor; and 16 rats served as controls. The blood was obtained from the rats' tails on the 4th, 6th, 8th and 10th day following the injection of the antigen, and the hemolysin titre determined. The results are shown in the table:

TYPE OF TREATMENT	NUMBER OF ANIMALS	4TH DAY	AVERAGE TITRES		
			6th day	8th day	10th day
Cortico-adrenal extract.....	16	2,300	5,130	2,015	355
Inactivated extract.....	16	1,030	2,710	1,085	215
Untreated controls.....	16	1,670	3,050	1,310	230

The hemolysin titres of the extract treated animals were 40 to 70 per cent higher than the untreated controls, and 70 to 130 per cent higher than the animals receiving inactivated extract.

In a series of 16 adult rabbits of the same litters, the immunization was studied over a period of 30 days. The animals received 5 cc. of a 10 per cent suspension of sheep cells intravenously every other day for a period of 20 days. The titre was studied every 4 days. The rabbits which received cortico-adrenal extract (8) produced an average titre 82 per cent greater than the controls on the 4th day; on the 8th day, the experimental animals' titre averaged 54 per cent higher; on the 12th day, it was 25 per cent higher; and at the 30th day, the titres were about equal in both experimental and control animals.

These experiments enhance the view that the adrenal glands are active in immunization and suggest the administration of cortico-adrenal extract to aid in the process of building immune bodies.

The effect of temperature on the rate of blood flow in the normal and in the sympathectomized hand. NORMAN E. FREEMAN (introduced by W. B. Cannon). Surgical Laboratories, Massachusetts General Hospital, and Harvard Medical School.

The rate of blood flow was determined in normal and in sympathectomized hands by a modification of the technique of Howlett and Van Zwaluwenburg. The hand was immersed in water in an insulated plethysmograph and the rate of arterial inflow was measured volumetrically after occlusion of the venous outflow. As the local temperature was raised the circulation was found to increase according to a curve which was constant for the individual under basal fasting conditions. With the normal hand

immersed in water at constant temperature, the rate of blood flow was found to be decreased when cold and increased when heat was applied to the body. In the sympathectomized hand the blood flow did not increase when the body was heated. When the body was chilled, the decrease in flow in the sympathectomized hand was ascribed to adrenal secretion. With the hand immersed in cold water, novocaine paralysis of the sympathetic nerves produced a rise in the blood flow on the injected side. Ten days after cervico-dorsal ganglionectomy, the blood flow was quite stable on the operated side over wide variations in local temperature. Six months later, however, characteristic changes in circulation were again present in the completely sympathectomized hand when the local temperature was altered. Under these circumstances, the logarithm of the rate of flow was shown to be a linear function of the reciprocal of the absolute temperature (Arrhenius equation). It was therefore argued that the temperature controlled the metabolism of the hand and that the blood flow in turn was modified by the tissue requirements. Bloodflow debts were accordingly established in sympathectomized hands by sudden occlusion of the circulation at constant temperatures. The excess flow of blood above the basal requirements at a given temperature after release of the occlusion (reactive hyperemia) was found to be mathematically equal to the product of the initial rate of blood flow at that temperature and the duration of the circulatory arrest. This precise relationship did not obtain with prolonged occlusion associated with the production of pain. The excess blood flow then far exceeded the theoretical value of the debt. It was concluded that the blood flow in the normal hand responded to the needs of the body for thermal regulation. After removal of the sympathetic vasomotor nerves, the circulation then responded to the metabolic requirements of the tissues. Under certain circumstances, additional physiological or pathological stimuli affected the rate of blood flow. The hypothesis first suggested by Roy and Brown was supported; namely, that the local control of the blood flow was mediated through the concentration of metabolites in the tissues.

The synthesis of fatty acid esters of glycerol by the intestine in normal and diabetic dogs. SMITH FREEMAN (by invitation) and A. C. IVY. Department of Physiology, Northwestern University.

These experiments, involving normal and depancreatized dogs, were undertaken to ascertain whether or not the intestine of the diabetic dog could synthesize fatty acid esters of glycerol from ingested fatty acids.

The general experimental procedure for normal and diabetic dogs included a 48 hour fast prior to cannulation, nembutal anesthesia, cannulation of the thoracic duct, collection of a sample of fasting lymph, and then feeding (by stomach tube) of an emulsion containing oleic acid, sodium oleate, bile salts and gelatin. Diabetic dogs were cannulated 5 to 10 days after removal of the pancreas; from the day of pancreatectomy until 48 hours before cannulation these dogs received 10 clinical units of insulin daily. A determination of blood and urine sugar values was made on each diabetic dog after cannulation. The completeness of pancreatectomy was verified by a postmortem examination of all dogs showing fat absorption. After fatty acid feeding the collection of lymph was continued for a number of hours. If there were visible signs of fat absorption, the fasting and fat-rich lymph was analyzed for fat-soluble ester glycerol; some

samples were also analyzed for total fatty acids, cholesterol and lipoid phosphorus.

Sixteen depancreatized dogs were studied. Of these six showed a definite increase in the fat-soluble ester glycerol in the lymph of the thoracic duct following fatty acid feeding.

These experiments have demonstrated an increase in the fat-soluble ester glycerol content in the lymph of normal and depancreatized dogs following the enteral administration of oleic acid. Undoubtedly, the synthesis of fatty acid esters of glycerol is effected by the intestine; and while such experiments do not prove that the diabetic dog can synthesize glycerol, such an inference appears justifiable from the results obtained.

The control of gastric hunger contractions in man by hypnotic suggestion.

H. L. FRICK (by invitation), R. E. SCANTLEBURY (by invitation) and T. L. PATTERSON. Department of Physiology, Wayne University, Detroit.

Since it has been demonstrated that the secretion of gastric juice in man may be augmented and its total acidity increased in hypnosis, it seemed to be of sufficient physiological importance to carry out a similar set of experiments to determine its influence on the tonus and contractions of the empty stomach. The balloon method was employed for recording these contractions, the balloon being introduced through the esophagus into the stomach.

The experimental evidence presented indicates that in light hypnosis the hunger contractions are inhibited promptly with the suggestion of food, while, on the contrary, the contractions are not as easily elicited with the suggestion of hunger when the stomach is in a state of quiescence. However, similar suggestions in the waking condition seem to influence the gastric hunger movements in no appreciable degree. It is apparent that the depth of the hypnosis determines the success of this procedure. We have also observed that the subject becomes restless when hunger is suggested which is typical of observations made during hunger in experiments on men and animals. These preliminary studies show that the process normally controlled by the autonomic nervous system may be influenced directly by suggestion.

Further evidence obtained is indicative that dreaming during normal sleep inhibits the gastric hunger contractions, the result of which is in confirmation with work already published on dogs.

The utilization of carbohydrate during aerobic activity in isolated frogs' muscles. C. L. GEMMILL. Department of Physiology, Johns Hopkins University, School of Medicine.

A study was made of the oxygen consumption and the disappearance of total fermentable carbohydrate during activity of frogs' isolated sartorii. The muscles were stimulated nine times a minute for periods of three to six hours in oxygenated Ringer's solution. The average utilization of carbohydrate accounted for only 42 per cent of the total energy exchange as obtained from the oxygen consumption. Therefore other material besides carbohydrate is oxidized to supply the energy for the contraction of isolated frogs' muscle under aerobic conditions.

The influence of temperature on parathyroid tetany in the albino rat.

VALERIA E. GENITIS, E. L. BORKON and R. D. TEMPLETON (introduced by T. E. Boyd). The Department of Physiology, Loyola University School of Medicine and the Department of Physiology, University of Chicago.

The thyroid and parathyroids were removed from 287 albino rats between the ages of 22 and 33 days, inclusive. The animals were from two colonies, one from the University of Chicago and one from Loyola University. The diet of the mothers consisted of Purina Fox Chow, ad libitum, with meat and bread once a week. The experimental animals received the same diet when weaned twenty-one days after birth.

Operated litter mates of the same sex and as nearly the same weight as possible, were distributed into three rooms. The temperature of the first was 80° to 93°F., the second, 63° to 80°F., and the temperature of the third, 54° to 70°F.

Observations were limited to 24 hours following the operation, during which time no food was given. At the end of this period the animals were classified as surviving without tetany, surviving but with tetany, or dead. Examinations for symptoms of tetany were made three times during the second 12 hours. Because of the difficulty in distinguishing mild degrees of tetany, a definite rigidity, developed in one or more limbs on handling the animals, was taken as the index and considered positive if it appeared at any one examination.

	NUMBER OF RATS	SURVIVING WITHOUT TETANY		SURVIVING WITH TETANY		DEAD	
		Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent
Warm room.....	85	15	17.6	19	22.4	51	60
Intermediate.....	137	29	21.2	89	65	19	13.8
Cool room.....	65	40	61.5	19	29.3	6	9.2

Several previous workers have noted incidentally that high temperatures were deleterious to parathyroidectomized animals. The above figures indicate that temperature does materially affect the incidence of serious tetany and especially the mortality rate.

Potentials associated with the respiratory act and their localization in the central grey axis stem. ROBERT GESELL, JOHN BRICKER (by invitation) and CONWAY MAGEE (by invitation). Department of Physiology, University of Michigan, Ann Arbor.

Action potentials associated with the respiratory act, recorded with the aid of fine needle electrodes inserted at various levels and depths in the brain and cord, have been described in a preliminary report in the February Proceedings of the Society for Experimental Biology and Medicine. Exploration for and recording of potentials have been made in thirty dogs. With the application of a weak polarizing current through the tips of the leading off electrodes, minute lesions were produced at the site of 31 sets of potentials in 10 animals. These lesions were localized histologically with serial microscopic sections.

Potentials, obviously related to the respiratory act, were most readily encountered in the medulla and upper cervical cord. They were less readily encountered in the lower thoracic and upper lumbar segments of the cord and as yet have not been met above the medulla.

In the cord, respiratory potentials have been obtained in the posterior and anterior columns, in the posterior funiculus at the medial edge of the posterior columns where the dorsal root fibers and their collaterals enter the posterior column and from the reticular formation.

In the medulla, respiratory potentials have been obtained in the ventro-mesial column, hypoglossal nucleus, ambiguus nucleus, in the course of the internal arcuate fibers just lateral to the hypoglossal nucleus, from the edge of the ventral reticulo-spinal tract, from the root fibers of the trigeminal and hypoglossal nerves, from Roller's nucleus, from nucleus intercalatus and possibly from nucleus eminentia teres, from the immediate vicinity of the median longitudinal fasciculus and median tecto-spinal tract, from the reticulo-spinal tract and from the medial and lateral reticular nuclei.

Present indications are that a diffuse arrangement, possibly extending through the reticular formation of the medulla and greater length of the cord is important in the nervous control of breathing. Further conclusions will be postponed awaiting a greater accumulation of records of potentials and their associated lesions.

Action potentials—central and peripheral. ROBERT GESELL, RONALD CULVER (by invitation), JOHN BRICKER (by invitation) and CONWAY MAGEE (by invitation). Department of Physiology, University of Michigan, Ann Arbor. (Demonstration.)

Action potentials, such as encountered in our studies on respiratory muscles, respiratory nerves and central grey axis stem, are recorded for sound reproduction.

Changes in the human electroencephalogram associated with loss of consciousness. F. A. GIBBS (by invitation) and H. DAVIS. Department of Physiology, Harvard Medical School.

The fluctuations in potential arising from the brains of human subjects have been studied with special reference to the manner in which they are altered during disturbances of consciousness. The conditions chosen for study were sleep, unconsciousness from nitrogen breathing, clouding of consciousness produced by overventilation, and epileptic seizures. The electroencephalograms were recorded with an ink-writing "undulator," supplemented by photographic records from a cathode ray oscillograph.

As standard leads, we used a needle thrust into the scalp at the vertex as grid lead, and a similar needle in the lobe of the ear as ground. The most constant and pronounced fluctuations observable in the resting subject with these leads have frequencies of from 10 to 20 per second, and attain a maximum of 60 microvolts. In sleep the frequency of these predominant waves decreases to between 1 and 5 per second; their amplitude may or may not decrease, depending on whether or not the subject shows, when awake, waves of large amplitude. In those who show small and rather fast waves when awake, the amplitude will increase as the frequency decreases.

If a subject becomes unconscious from breathing nitrogen, the frequency

of his predominant waves decreases to between 1 and 5 per second, and the amplitude increases to about 100 microvolts. This change occurs rather gradually, beginning definitely before the subject loses consciousness.

If the subject overventilates his lungs sufficiently to produce clouding of consciousness, the same alterations in the electroencephalogram occur.

An epileptic seizure is associated with the sudden appearance of large slow waves, having a frequency of 1 to 5 per second and an amplitude of more than 100 microvolts. These waves may amount to as much as 500 microvolts.

After nitrogen breathing, overventilation, and epileptic seizures, with their attendant big, slow waves, there is a decrease in all electrical activity; then a gradual return of the normal waves.

Toxicity of two vital dyes used in plasma volume determinations. J. G. GIBSON, 2nd (by invitation) and MAGNUS I. GREGERSEN. Laboratories of Physiology and the Department of Medicine, Harvard Medical School.

Two vital azo dyes: Brilliant Vital Red (N.A.C.) and T-1824 were found non-toxic for rats in doses of 20 mgm./kgm.; growing male litter mates receiving this amount intravenously showed no deviation in growth rate from that of controls injected with normal saline. Animals injected with 45 mgm./kgm. suffered a decreased rate of growth, greater for those receiving T-1824 than Brilliant Vital Red, but all animals survived the experiment (87 days).

Study of necropsy material disclosed the dye intra-cellularly in renal epithelium and hepatic parenchyma but no necrotizing effect was observed. These dyes do not pass the renal glomerulus or choroid plexus. They are removed from the circulation 1, by the liver via the biliary tract, and 2, by storage in the reticulo-endothelial system. Constant findings in animals receiving the larger dose were multiple pulmonary embolic processes, with an increased number of phagocytes in the lung parenchyma, and hemosiderosis of the spleen; these effects indicate that the chief danger to be anticipated from large intravenous doses is in pulmonary emboli.

T-1824 has been given intravenously in doses of from 0.15 mgm./kgm. to 0.2 mgm./kgm. to fifteen human subjects without subjective or objective untoward effect.

The differences in voluntary water intake following the intravenous administration of hypertonic NaCl and urea. ALFRED GILMAN. Department of Pharmacology and Toxicology, Yale University. (Read by title.)

Unanesthetized, trained dogs were injected intravenously with isosmolar solutions (2.5 cc. per kilo) of either hypertonic NaCl (20 per cent) or hypertonic urea (40 per cent). Blood samples were taken before injection, 30 minutes thereafter, and in some experiments at intervals for six hours. Water was given *ad lib.* after the second blood sample had been withdrawn, and the voluntary water intake followed for 24 hours. Changes in blood osmotic pressure and specific gravity were measured.

The increases in blood osmotic pressure following the injection of either the hypertonic urea or sodium chloride solution were identical. However, the saline administrations were accompanied by a marked shift of fluid from an intra- to extra-cellular environment, as evidenced by the pronounced decrease in the specific gravity of the blood serum. No such

fluid shift followed the urea administration. When given access to water, the animals receiving saline greedily drank, within an hour, sufficient to return the concentration of their body fluids back to normal. The animals receiving urea showed much less thirst, drank sparingly, and their osmotic pressure remained elevated over a period of six hours. Inasmuch as both groups of animals showed an identical rise in osmotic pressure, the inference is drawn that the primary thirst stimulus evidenced by those animals receiving NaCl was a cellular dehydration rather than the general increase in the concentration of osmotically active substances common to both groups.

Vagus inhibition and interference with conduction of the cardiac impulse.

A. S. GILSON. Physiological Department, Washington University School of Medicine.

In the atrium of the turtle (genus *Pseudemys* and genus *Chrysemys*) vagus inhibition results *inter alia* in a reduction of strength of beat, a shortening of the absolutely refractory period, and a delay or prevention of the return to "normal threshold" as determined by the use of rheobasic constant currents or double condenser shocks of long duration. Similar effects are seen in the sinus but in the ventricle no such effects are observed.

The vagus threshold (shock intensity or frequency) for the appearance of vagal sino-atrial block is close to the thresholds for sinus inotropic depression and slowing of heart rate. Atrial inotropic depression appears at a lower vagus threshold than does sino-atrial block. Simple vagus atrioventricular block has not been observed in the turtle although it is easily produced in the frog heart. In the frog heart there is a marked vagal inotropic effect on the ventricle.

Determination of thresholds to long duration shocks yields data regarding the functional distribution of the vagus in the turtle heart which correlates perfectly with the functional changes observed by other means.

It is considered probable that in these experiments, block of impulse conduction between one chamber and another is normally associated with vagus depression in both chambers. Preparations having depressed junctival regions may show junctival block when only one of the chambers is affected by vagus inhibition.

Changes of the RQ following the injection of methylene blue, cysteine, or cystine. WALTER GOLDFARB (by invitation) and H. E. HIMWICH. Yale University, New Haven.

There are few observations on the rôle of dehydrogenases in the living animal. We have previously reported changes which occur in the metabolism of rats after injections of methylene blue. These experiments have been extended to include cysteine and cystine, dehydrogenases normally present in the body. Rats were used as experimental animals in the Haldane open circuit system. Metabolism determinations were made after a 24-hour fast. The animals were then injected subcutaneously with either 1 cc. of M/100 cystine, 0.5 cc. of M/100 cysteine, or 0.5 cc. of M/100 methylene blue per 100 grams of body weight. The initial effect observed was a depression of the quotient which reached levels as low as 0.55 and persisted as long as 50 hours in some cases. This was followed by a secondary rise which always exceeded the quotient of the foreperiod, and in some instances exceeded 1.0. The quotient then fell gradually to

the original level of fat. The respiratory quotient of the entire experiment approximated that of fat oxidation. The average of 15 experiments was 0.709 ± 0.007 . This indicated that the substance mainly oxidized was fat. Two explanations of the intermediary processes of oxidations might be suggested. Fat might first be converted to carbohydrate, resulting in the initial fall of the quotient, followed by the oxidation of the carbohydrate in the second phase. In order to test this hypothesis, methylene blue was injected into phlorhizinized rats. If fat was converted to carbohydrate, the glucose would be excreted and the secondary rise of the quotient would not appear. In these experiments the earlier data were closely duplicated indicating that the conversion of fat to carbohydrate did not occur. On the other hand, the hydrogen acceptors might remove hydrogen from some substrate in the body, perhaps fat or carbohydrate. The rate of water formation would exceed that of CO_2 formation and cause a fall in the quotient. The subsequent oxidation of the remainder would raise the quotient in the second phase. At present we are attempting to elucidate the nature of this hypothetical substrate.

Agents modifying the effect of subthreshold induction shocks. HELEN TREDWAY GRAHAM. Department of Pharmacology, Washington University School of Medicine, St. Louis.

The second phase (post-cathodal depression, post-anodal enhancement) of the effect of a subthreshold induction shock on the irritability of an isolated frog sciatic nerve may be markedly increased by painting the nerve with Ringer's solution containing excess K^+ ions (3 times the normal K^+ concentration). Nerves moistened with normal Ringer's solution do not always manifest the second phase, and when they do the maximum change of irritability during this period rarely exceeds 1 to 2 per cent; nerves treated with excess potassium always manifest the second phase, and the maximum change of irritability may be 5 per cent or more. The duration of the phase also tends to be increased. Post-cathodal depression is likewise increased under the influence of cocaine hydrochloride or amyl alcohol. Excess Ca^{++} ions (9 times the concentration in normal Ringer's) decrease or prevent the manifestation of the second phase. The first phase is shortened when the second phase is increased, and usually lengthened when the second phase is decreased. A mild degree of poisoning with either veratrine or yohimbine does not seem to produce any significant change in the subthreshold effect.

Grouped according to their influence on the resting level of irritability or on the after-potentials, these agents fall into groups different from those determined by their influence on the second phase of the subthreshold effect; their influence on the relatively refractory period seems however to be consistently parallel with their influence on the second phase of the subthreshold effect.

Further attempts to evaluate phasic changes of coronary flow by means of differential pressures. H. D. GREEN (by invitation), D. E. GREGG and CARL J. WIGGERS. Department of Physiology, Western Reserve University Medical School, Cleveland, O.

The experiments of Gregg, Green and Wiggers (these Proceedings) seemed to validate a method for evaluation of phasic changes in coronary flow by use of differential pressure curves.

Determinations of the volume backflow per systole from a coronary branch, combined with volume elasticity studies (a) of the coronary system, and (b) of the manometer showed however that there was less fluid moved back into the superficial vessels and hence into the optical manometer than was required to register the full magnitude of the pressure developed.

Consequently such peripheral coronary pressure curves, while showing the contour and relative pressure changes, would, when subtracted from central coronary pressure curves, give an exaggerated systolic onflow. They are therefore not allowable unless corrected as to true ordinate values.

To determine then the true magnitude of the peripheral coronary systolic pressure, the coronary artery was electromagnetically clamped for short intervals, at a rate slightly faster than the heart rate, as suggested by Anrep and Saalfeld, while the pressure was recorded from a side branch peripheral to the clamp. Thus fluid could be trapped in the coronary system at varying pressures up to aortic systolic pressure. The level at which the pressure in the coronary system just did not fall during systolic clamping was taken as the critical value for systolic peripheral coronary pressure.

The lowest diastolic pressure recorded during electromagnetic clamping lasting for two beats was used as the value for diastolic pressure.

By redrawing the previously obtained peripheral coronary pressure curves with these new ordinates and subtracting them from central coronary pressure curves, differential pressure curves were obtained which show that a systolic as well as a diastolic intramural onflow occurs and that the total systolic flow is slightly less than during an equivalent interval of early diastole, but distinctly less than during a similar interval later in diastole.

Evidence of superior cervical pathways for efferent coronary dilator neurones in the dog. C. W. GREENE. Department of Physiology and Pharmacology, University of Missouri.

At the last annual meeting experiments were reported showing the distribution of coronary constrictor neurones obtained by the method of degeneration of the vagus following section of the vagus trunk central to the ganglion nodosum in the dog. This operation leaves the vago-sympathetic trunk completely denuded of cardiae inhibitory nerves, though Goland and Morgan have indicated that some inhibitory response follows stimulation of the ganglion nodosum.

The striking point that does appear after vagus degeneration is the presence of coronary dilator reactions to stimuli applied to this pathway in the region between the superior and inferior cervical ganglia. These neurones are not present in every individual dog tested, showing animal variations. They are apparently present in greater numbers in the lower cervical region, i.e., immediately above the inferior cervical ganglion. In some animals stimulation of the superior cervical ganglion itself induces coronary dilatation.

Enterogastrone. H. GREENGARD (by invitation), J. S. GRAY (by invitation) and A. C. IVY. Department of Physiology, Northwestern University Medical School, Chicago, Illinois.

Experiments in this laboratory and that of Lim have shown that the

intestinal mucosa yields material which will suppress the motor and secretory activity of the stomach. Crude preparations are contaminated with much protein and other substances which are definitely toxic. Separation of these is accomplished by trituration of the fresh mucosa with picroic acid, extraction of the mixed pierates with acid alcohol, reduction of the extract in vacuo to a small volume, and precipitation with a large amount of acetone. This is essentially the same as the procedure of Lim, Ling and Lin. We have effected further purification by solution of the crude material in water, ultrafiltration through a cellophane filter, reprecipitation with picroic acid, and repetition of the acid alcohol extraction and acetone precipitation. The product is colorless, readily soluble in water, and non-toxic. It is free of vaso-depressor activity and does not contain secretin or cholecystokinin.

An achlorhydria of one to five hours' duration has been obtained in dogs, which in some cases was evidenced as a complete achylia. The inhibition of motility is somewhat less constant in duration, but in general a cessation of motility has been found to occur, lasting from thirty minutes up to over three hours.

Plasma volume determination with dyes: errors in colorimetry; use of the blue dye T-1824. MAGNUS I. GREGERSEN, J. J. GIBSON (by invitation) and E. A. STEAD (by invitation). Department of Physiology, Harvard Medical School.

Correct estimation of the plasma dye concentration is made difficult by 1, the inherent color of the plasma; 2, presence of hemoglobin; 3, lipemia, and in repeated determinations 4, the residual dye. In methods employing a simple colorimeter, the direction and magnitude of the error from 1 and 4 depend upon the relation between the concentration of dye in the standard and the unknown, i.e., upon the strength of standard which happens to be used. If the residual dye is large in amount, and is ignored (for method of correction see H. P. Smith, 1920), the apparent plasma volume may easily be as much as 25 per cent above or below the true value. Theoretically the compensating colorimeters should correct for 1 and 4 but slight turbidity frequently gives rise to bizarre results. Moreover, if there is hemolysis in either control or dye-sample a determination cannot be made. Spectral absorption curves of various vital dyes in water, saline and plasma solutions show the errors introduced in plasma volume determinations by using water or saline instead of plasma for preparation of standards.

Spectrophotometric analysis presents several advantages. The measurements of color strength are made in absolute terms (optical density) so that no comparison standard is required. The absorption due to the solvent, lipemia or residual dye is cancelled by measuring directly the difference in optical density between control- and dye-sample. When using red dyes, corrections must be made for errors caused by hemolysis (Graff and Clark, 1931). But, if one employs a blue dye such as T-1824 (first obtained from Dr. H. M. Evans and later synthesized by Dr. L. F. Fieser and Mr. Hartwell (Harvard)), hemolysis can be ignored (practically) since hemoglobin adds relatively little to the optical density of the sample at 620 to 630 mu, the range of maximum absorption of this dye in plasma solution.

The slow disappearance rate of T-1824 (5 to 8 per cent per hour), ob-

served by Dawson, Evans and Whipple (1920), has been confirmed in numerous experiments in the dog and in man.

Centrifugation (30 minutes at 3000 rpm.) of blood containing a known amount of T-1824 leaves 4 per cent of the dye unaccounted for in the plasma above the cells. Since coagulation of plasma or whole blood fails to remove T-1824 from solution (Gregersen and Sehro, unpublished observations), and since serum samples yield dye values 3 to 5 per cent higher than those obtained by the plasma technique (involving addition of isotonic anticoagulant and depending upon the hematocrit for the correct dilution factor), it appears that the 4 per cent loss of dye is due to the trapping of plasma among the cells after centrifugation, and not to absorption of dye by the red cells.

Twice the amount of T-1824 required for a single determination in man (10 to 15 mgm.) does not produce visible staining of either skin or sclera.

Determinants of peripheral pressure pulses in a coronary ramus. D. E. GREGG, H. D. GREEN (by invitation) and C. J. WIGGERS. Department of Physiology, Western Reserve University Medical School, Cleveland, O.

We suggested recently (Proc. Nat. Acad. Sciences, Science 80: 545, 1934) that phasic changes in coronary flow may be evaluated by combining calibrated peripheral and central optical coronary pressure curves. To establish the applicability of the method it is essential to analyze the factors that determine such peripheral pressures. The importance of collateral pressure, myocardial shortening and myocardial tension were therefore investigated.

Normal peripheral coronary pressures were recorded optically from a large lateral branch of the left anterior coronary artery during temporary clamping of the vessel central to the branch. Such curves were compared (a) with those obtained after clamping the right coronary, the left circumflex or both, and (b) with those obtained from the area, when optical myograms (Tennant, these Proceedings) showed systolic lengthening instead of shortening.

Results: 1. The peripheral coronary pressures are not affected materially by collateral pressure transmission because (a) the steep and major rise of pressure is attained during the isometric contraction period; (b) clamping either the right coronary or left circumflex produces no phasic alterations of such curves which cannot more logically be attributed to concurrent altered dynamics of ventricular contraction. 2. The replacement of normal myocardial shortening by systolic extension in an ischemic area does not alter materially the form, amplitude or phasic relationship of the peripheral coronary pressure curves. 3. The steep rise and fall of peripheral coronary pressure occurs prior to muscle shortening and lengthening and simultaneously with the steep rise and fall of intraventricular pressure. Thus it appears probable that peripheral coronary pressures are controlled largely by muscle tension changes.

Further studies on the adrenal cortical hormone. ARTHUR GROLLMAN and W. M. FIROR. The Johns Hopkins Medical School.

A relatively simple method has been devised for obtaining the adrenal cortical hormone in a form suitable for oral administration. The method is based on the finding that the hormone is readily adsorbed by charcoal

from a neutral aqueous solution, from which combination it is eluted in the gastro-intestinal tract. Adrenal glands are extracted with acetone and the acetone is removed by distillation *in vacuo*. The neutralized aqueous residue is agitated with charcoal and filtered. The charcoal-hormone combination thus obtained is washed free of adsorbed noxious impurities. When administered to bilaterally adrenalectomized dogs in 2 to 4 gram doses daily, the charcoal-hormone combination manifests complete replacement therapy. One-month-old adrenalectomized rats to whose food was added 0.1 gram of the charcoal-hormone combination grew normally to adult size.

The administration of physiologically active substances (difficult to prepare in a pure form suitable for parenteral injection) in the form of an adsorption compound is of general application wherever the substance is eluted by the gastro-intestinal tract and is not readily destroyed by the gastro-intestinal juices. We have utilized an analogous procedure for administering the estrogenic substance of urine which (as previous workers have shown) is readily adsorbed by charcoal. Administration of the charcoal combination is an effective means for eliciting the estrogenic reaction and avoids the necessity of preparing purified extracts.

Adrenalectomized young rodents treated with an adequate dose of the adrenal cortical hormone grow and develop normally in every respect. The development of a resistance to therapy, such as has been described by previous observers, is due to inadequate therapy. The chronic adrenal insufficiency induced in this way, as well as by other experimental methods, leads to cessation of growth and the development of symptoms resembling hypophyseal cachexia. Injection of extracts of the growth hormone of the pituitary will cause the resumption of growth in these animals while treatment with adrenal cortical hormone is without any effect. Anatomical findings also indicate that a secondarily induced pituitary insufficiency is responsible for many of the symptoms observed in chronic experimental adrenal insufficiency.

Some effects of hydrostatic pressure on nerve action potentials. HARRY GRUNDFEST and MCKEEN CATTELL. Department of Physiology, Cornell University Medical College, New York City.

With the aid of the cathode ray oscillograph a preliminary study has been made of the changes produced by increased hydrostatic pressure on the activity of frog medullated nerve (sciatic). The following effects have been observed for moderate pressures (5000 to 8000 pounds per square inch).

Under pressure a single shock results in a second response in fibers of lowered threshold, giving rise to a well marked elevation from about 1.3 to 2.8σ after the start of the conducted A wave. A lowering of the threshold of excitation occurs beginning with pressures of about 1500 pounds. The repetitive responses occur, however, only with pressure above 4000 pounds. The intensity of the stimulus appears to play no part in the production of repetitive responses since they are observed in single fiber responses to a threshold stimulus. Two or more repetitions are frequent. Pressure results in a slight increase in the height of the action potential, and this is particularly true of the β and γ elevations, which become more pronounced. The whole curve is spread out, resulting in a considerable increase in total area. Measurements of single fiber responses commonly show a similar

change, the height being increased about 10 per cent and the duration 20 per cent. Under pressure there is an increase in conduction velocity of from 8 to 15 per cent. Both the absolutely and the relatively refractory periods are prolonged.

With higher pressures (8000 to 15,000 pounds per square inch) the size of the action potential is decreased, the threshold greatly increased, and the conduction rate markedly slowed. All these changes are completely reversed with the release of pressure. Immediately following release of pressure there is a marked increase in threshold, which subsides after a few minutes.

Further studies on amino acids in development. VI. The relative efficacy of single amino acids at different periods. F. GUDERNATSCHE and O. HOFFMAN (by invitation). New York University, Washington Square College, New York City. (Read by title.)

During development an organism's needs for its constituents vary. In studying the value of single amino acids at different stages, Rana sphenoccephala larvae were reared on normal diet for 1, 4, 6 weeks resp. (sets I, II, III), then given a non-nitrogenous basal diet plus one acid in 1:1000 solution. Experimental period 182 days.

Set I. Insufficiency noticeable by 56th, acute by 67th day. Alanine and arginine groups showed best maintenance. Some starvation controls reached 66th, basal diet 128th, leucine, alanine, arginine, tryptophane, histidine 182nd day. With little variation and slight increment above basal diet, best early growth in arginine, alanine, proline, poorest in glutamic. Latter did not exceed starvation. Best 86th day length increments in arginine, leucine, tryptophane, phenylalanine. No differentiation.

II. Best maintenance throughout in alanine, glutamic, arginine, phenylalanine, proline. Insufficiency evident by 67th, acute by 103rd day. Some starvation controls reached 101st, basal diet 138th, the following 182nd day: 5 per cent alanine, cysteine; 10 per cent leucine, glutamic, arginine, phenylalanine, tyrosine, histidine; 15 per cent tryptophane; 20 per cent proline. Best growth in alanine, cysteine, tryptophane. Glutamic, proline quite good. All groups except glycine, arginine and phenylalanine exceeded basal diet by 56th day. Differentiation in increasing degree: cysteine, glutamic, histidine, slight; phenylalanine, aspartic, proline, largest animals; tyrosine, early; tryptophane, all animals showing signs of continued development.

III. Best maintenance throughout: leucine, arginine, histidine. Insufficiency definite by 90th, acute by 123rd day. Starvation reached 116th, basal diet 139th, the following 182nd day: 15 per cent phenylalanine; 20 per cent glycine, leucine, arginine; 25 per cent alanine, histidine. Best growth in leucine, aspartic, glutamic, phenylalanine, tyrosine, histidine, though no increment after 48th day. No differentiation beyond hind limb stage. After 46 days tyrosine, proline, aspartic (large size) most advanced, arginine least; after 163 days best differentiation in arginine, phenylalanine, tyrosine, tryptophane, aspartic, histidine.

In general: maintenance values increased with increasing normal diet periods. Progressive growth most frequent in I. In the older animals the acid concentration may have been insufficient for tissue building or specific demands for protein components may become more selective once the

basic pattern is formed. Differentiation, naturally, improves with length of normal feeding period.

Further studies on amino acids in development. VII. The relative efficacy of amino acid mixtures at different periods. F. GUDERNATSCH and O. HOFFMAN (by invitation). New York University, Washington Square College, New York City. (Read by title.)

When *Rana sphenoecephala* larvae were fed on normal diet for 1, 4, 6 weeks resp., then on basal diet plus one amino acid, the sets showed differences in maintenance, growth, differentiation. We then tested the physiological value of 2 to 13 acid mixtures. Experimental period 182 days.

Set I. Maintenance numbers in two acid mixtures highest in leucine-tyrosine, in three acids in leucine-arginine-tyrosine. Maintenance span good in leucine-cysteine, also in leucine-arginine-tryptophane, the only higher mixture reaching 182nd day. Greatest length in leucine-cysteine, next in cysteine-tryptophane. Arginine-leucine and arginine-tryptophane did not exceed basal diet which barely showed growth. Some 3-, 6- and the 9-acid groups showed good early growth. The 13-acid group died early, without having exceeded starvation group. Differentiation was positive in leucine-tryptophane and leucine-arginine-tryptophane.

II. Maintenance numbers better than starvation and basal diet, outstanding in leucine-cysteine and nine acids. Longest spans in leucine-cysteine, leucine-tyrosine, arginine-tyrosine, arginine-tryptophane. Life span in higher mixtures usually shorter, slightly better than in I. No higher mixtures reached 182nd day. Growth after 56 days in almost all exceeding starvation and basal diet. Leucine-arginine-tyrosine was best. No differentiation in starvation, barely noticeable in basal diet, some in several groups, most progressive in arginine-tryptophane.

III. Maintenance below that of II. Insufficiency definite by 123rd day. Longest spans in arginine-leucine and arginine-tyrosine. Growth continued somewhat, though almost none after 102nd day. Some six acid groups were good, also leucine-cysteine, cysteine-tryptophane. Differentiation well started when acid feeding began but little further progress. Arginine-tyrosine advanced most.

Former experiments (without initial normal diet periods) showed that the value of amino acid diets (1 to 13 acids) usually improves with the complexity of the mixtures. In the present experiments more complex mixtures often proved of less value than simpler. Insufficient concentration may have been the factor. Or, changing from the natural to an artificial diet may have a more acute effect than the latter alone, for protoplasm built up by complete diets may have more specific maintenance requirements.

The effect of exercise on the respiratory exchange and some urinary constituents following the ingestion of glucose and fructose, separately and in combination. JOHN HALDI and GEORGE BACHMANN. Laboratory of Physiology, Emory University School of Medicine.

To determine what effect the ingestion of the sugars mentioned in the preceding communication would have on the respiratory exchange of exercise and recovery, the same two subjects performed 550 kgm. of work per minute for $\frac{1}{2}$ hour on a bicycle ergometer, immediately following the

ingestion of (a) 500 cc. water at 37°C., and (b) 50 grams of the sugars separately or in combination dissolved in the same volume of water.

During the first 15 minute period of exercise, the R.Q. rose from an average basal level of 0.79 to an average of 0.89 and remained at approximately the same height in the second 15 minute period, whether water or any of the sugars were ingested. During the first period of recovery, the R.Q. of one subject, in the experiments with water, rose to 0.92, that of the other subject dropped to 0.88 and then both fell rapidly in the next two periods, reaching the basal level at the end of the third period. In the experiments with sugar, the R.Q. rose during the first recovery period to a higher level with each subject. With one subject it rose to 0.95, 1.01 and 1.10 with glucose, fructose and their combination respectively; with the other subject it rose to 0.95, 1.01 and 1.03 respectively. The R.Q. then dropped abruptly in the second recovery period and, in the experiments with fructose and combination of the two sugars, continued to fall gradually to the end of the experiment. In the glucose experiments the R.Q. remained at a fairly constant level during the last four 15 minute periods. In none of the experiments with sugar had the R.Q. returned to the basal level 1½ hour after exercise.

The same urinary constituents were determined at the end of the basal and recovery periods as in the preceding experiments. In 3 out of 6 cases, exercise and water led to the excretion of creatine, whereas in 20 out of 23 experiments with exercise and sugar, creatine appeared in the urine after exercise. There is some indication that more creatine was excreted in the experiments with fructose. The total nitrogen elimination per hour was invariably less after exercise. There is also some indication of an increase of the titratable acidity of the urine after exercise in the experiments with fructose.

Optical tracings of human blood pressure, comparing the clinical and oscillographic criteria with the true blood pressure. W. F. HAMILTON, R. A. WOODBURY (by invitation) and H. T. HARPER, JR. (by invitation). Department of Physiology and Pharmacology and the Department of Medicine, University of Georgia, School of Medicine, Augusta, Georgia.

The manometer described in a previous communication has been perfected so that it gives a nearly aperiodic and very quick deflection when equipped with a 22 to 26-G needle. With these very small needles it is a simple matter to enter the human brachial artery and to record the pressure pulses. These can be registered continuously. Slides will be shown in which the systolic and diastolic pressures are measured and compared with the auscultatory and oscillographic criteria. Clinical cases with various cardiovascular diseases have been recorded and the true blood pressure compared with the results of interpreting the indirect criteria. Continuous tracings have been made in which the blood pressure changes can be followed during certain physiological activities such as normal, deep and abdominal breathing, straining and recovery and occlusion of arteries. The results of the administration of certain drugs have been followed on the human.

Impulses in single optic nerve fibres of the vertebrate retina. H. K. HARTLINE. Johnson Foundation, University of Pennsylvania.
Oscillographic records of action potentials in single optic nerve fibres

of the frog's eye have been obtained from small bundles of fibres dissected from the anterior surface of the retina near the head of the optic nerve. The discharge of impulses in such fibres, in response to illumination of that portion of the retina from which they come, shows considerable diversity, even among fibres from closely adjacent areas of the same eye. In certain of these fibres the response is similar to that in the optic nerve fibres of Limulus, showing an initial burst of impulses at high frequency, with subsequent adaptation to a lower level of steady discharge, which is maintained as long as the illumination lasts. In other fibres, however, only the initial burst is present, and the discharge is not maintained. Such fibres may give a slight response when the light is turned off. Another group of fibres has been found in which the initial burst is absent, and the discharge builds up relatively slowly (over one or two seconds) to its steady value. Such fibres also give an off effect. In the fourth group of fibres no response at all occurs during illumination, but as soon as the light is turned off a vigorous discharge occurs, initially at high frequency, but gradually dying out. In some cases this discharge may take many minutes to disappear, and occasionally has been observed to break up into a series of rhythmic bursts of impulses. Re-illumination promptly inhibits the discharge in this group of fibres.

It is supposed that this diversity of function among the optic nerve fibres of the frog retina is due to a modification of the original sensory discharge by the neural elements intervening between the sense cells and the optic nerve fibres.

The influence of exercise on water diuresis. FRANCES A. HELLEBRANDT.
University of Wisconsin Medical School.

Mild and moderate grades of exercise are without influence on water diuresis in man. Severe muscular work promptly suppresses kidney function as evidenced by oliguria. The inhibitory effect of a three minute bout of violent exercise is conspicuously apparent for one hour. There is a characteristic lag before the maximum depression of excretory rate is attained. This is reached 40 to 60 minutes post-exercise, and the basal excretory rate of the unstimulated kidney is then approximated. At the end of one hour urine formation abruptly increases and a typical diuresis curve follows. The maximum rate of excretion attained during the delayed diuresis may exceed normal.

We have been unable to abort this prolonged suppression of kidney function induced by exercise. Its mechanism is being studied along four different lines: 1, delay in absorption; 2, excessive production of the anti-diuretic principle; 3, post-exercise negative phase in blood pressure; 4, the lactic acid acidosis concurrently induced. We believe that water is being absorbed but not gotten rid of. Dilution of the anti-diuretic principle by the administration of large doses of water does not hasten the restitution of diuresis. This comes on before circulatory recovery is complete but the blood changes related to lactic acid accumulation subside at about the same time kidney function returns. Brief violent exercise is unassociated with chloride loss in consequence of profuse sweating, but the findings suggest that water intoxication may theoretically follow by virtue of a relative instead of an absolute diminution in salt concentration.

A study of the plasma concentration of the dye Wasser Blau under various conditions which alter plasma volume. ALLAN HEMINGWAY and F. BERNHART (by invitation). The Medical School, University of Minnesota, Minneapolis.

Wasser Blau is a non-toxic acid tri phenyl methane dye. After intravenous injection in dogs it is known to be present for several weeks in the plasma. The excretion rate of the dye from plasma has been measured in a series of five dogs over a 12 day period and the results show remarkable uniformity. The excretion during the first five days is exponential with time but after 5 days the excretion is linear with time. Repeated hourly sampling from normal trained unanesthetized dogs 6 to 7 days after dye injection shows a decrease in dye concentration greater than the theoretical amount as given by the normal excretion curve. The changes in dye concentration after saline injection, 5 to 10 days after dye injection, were measured with and without the visceral arteries clamped. The purpose of the observations is to determine if this dye is suitable for the measurement of relative plasma volume.

The thermo-stromuhr method of measuring blood flow. JULIA F. HERRICK, HIRAM E. ESSEX, EDWARD J. BALDES and FRANK C. MANN. Division of Experimental Medicine, The Mayo Clinic, Rochester, Minnesota. (Demonstration.)

The thermo-stromuhr of Rein has been modified so as to permit quantitative measurements of blood flow in any blood vessel of the normal intact dog. The units applied to the blood vessel are sterilized by boiling when the blood flow observations are to extend over several days. When the observations are limited to seven or eight hours the units are sterilized with an antiseptic. All dogs are trained to lie quietly on the table during blood flow observations. Units are placed on deep-seated vessels under ether anesthesia employing the usual sterile technique. Unless the study of the effect of ether anesthesia is desired no observations on blood flow are made until the dog has recovered completely from the effects of the anesthesia.

The thermo-stromuhr method of measuring blood flow has been completely described in a previous communication published in Physics, 1: 407-417, December 1931.

The method has been used for a period of four years and a series of investigations has been made such as the effect on the blood flow of 1, certain anesthetics; 2, the feeding of desiccated thyroid gland extract; 3, unilateral lumbar sympathectomy; 4, digestion of food, and 5, certain drugs. Many measurements have been made on superficial vessels such as the femoral vein, femoral artery, jugular vein and carotid artery. Observations on blood flow also have been made on the following deep-seated vessels: superior mesenteric, vena cava, renal arteries and veins, aorta and coronary arteries. Observations have been continued for as many as eighteen consecutive days, during which time the dog has been allowed to walk about freely, eat voluntarily, and behave normally.

Charts and moving pictures will give more detail concerning the manufacture of the units and the application to the vessels *in situ*, the condition of the experimental animal, the nature of the experiments and the results.

The technique employed in measuring blood flow will be demonstrated.

Changes in surface and rectal temperatures in man produced by the ingestion of food subsequent to twenty-four hour fast. J. F. HERRICK and CHARLES SHEARD. Division of Biophysical Research and Institute of Experimental Medicine, The Mayo Foundation and The Mayo Clinic, Rochester, Minnesota.

Eight thermocouples were attached to various areas of the surface of the body and remained in place during the course of any series of tests. Continuous automatically recorded data regarding rectal temperatures were obtained by a resistance thermometer. The persons who submitted to the test fasted for twenty to twenty-four hours. The temperature of the room in which tests were made was kept at 25° to 26°C. throughout these investigations. After the readings obtained from the thermocouples and rectal thermometer showed constancy per se within 0.5°C., or less for a half-hour, the subjects were fed a meal of orange juice, bacon, eggs, toast, and coffee. In general, these investigations show that there is an increase of 0.5° to 1°C. in the temperature in superficial areas situated at the thigh, axilla, upper arm, over the stomach, and on the forehead, following the ingestion of food. In the various persons who submitted to the test, the temperatures of the plantar surfaces of the large toes increased 2° to 13°C., during the eating of the meal (completed in ten to fifteen minutes) or within half an hour to an hour after its ingestion. From two to five hours after eating, the toes showed total increases in temperature, ranging from 3° to 15°C. The dorsa of the hands showed increases of 1° to 3°C., during the first half-hour to one hour after the subject had partaken of food, with increases of temperature ranging from 2° to 4°C., during the second to fifth hour. In general, the blood pressure increased 5 to 15 per cent and the pulse rate increased 10 to 20 per cent shortly after the subject had eaten. These effects disappeared within about an hour. The rectal temperatures increased in the range of 0.1° to 0.5°C., following the ingestion of food and during the course of digestion. The effects of various types of diet and environmental temperatures are being investigated.

Secretion of ammonia by the intestine. R. C. HERRIN. University of Wisconsin Medical School.

Studies on the chemical composition of succus entericus secreted in response to distention of jejunal fistulae in dogs demonstrated that after the appearance of anorexia the fixed base of the juice decreased 5 to 27 per cent and the ammonia in four animals increased to a concentration of 72 to 148 mgm. per cent. The ammonia content of the normal juice in these dogs was less than 2 mgm. per cent but in later experiments it was found extremely variable ranging from 4 to 27 mgm. per cent.

The fixed base conservation achieved by the secretion of ammonia suggested that a reduction of the available base was the exciting factor which was tested by the production of acidosis in two normal dogs with jejunal fistulae by fat feeding and in two others by a milk and CaCl_2 diet. In the case of one dog in each group, not only was there a reduced serum CO_2 capacity but also a 3.0 and 5.3 per cent reduction in serum base. In all cases the ammonia content of the intestinal juice showed a reduction of 16 to 80 per cent instead of the expected increase. The absence of response to acidosis, led to a consideration of blood urea. In two experiments performed on two dogs saline diuresis caused a 35 to 77 per cent reduction of blood urea and a 2.5 to 40.0. per cent decrease in the juice ammonia.

In four experiments on two dogs the intravenous injection or oral ingestion of urea resulted in a decrease of juice ammonia in two cases and a 41 to 173 per cent increase in the others. The evidence was not convincing for urea as a primary factor. A diet of sucrose and starch in seven animals decreased the juice ammonia from an average of 27.5 mgm. per cent to 8.5 which was raised to 40.6 mgm. per cent by a protein diet of lean beef. The results obtained with the fat, carbohydrate and protein diets indicate that the content of ammonia in the intestinal juice depends upon the rate of protein catabolism.

Studies on the pial vessels of the rabbit. ALRICK B. HERTZMAN and FLORENT E. FRANKE. Department of Physiology, St. Louis University School of Medicine, St. Louis, Missouri.

It seemed desirable to have information on the reactivity of the pial vessels of rabbits in comparison with those of other species and in anticipation of studies on the responses of these vessels to normally induced variations in the activity of the animal. These vessels have been studied by means of simultaneous observation and microphotography through specially designed cranial windows inserted in the parietal region of the rabbit under light morphine narcosis.

The pial arteries of the rabbit are very sensitive to thermal, mechanical and chemical influences, constricting to epinephrine and alkali, markedly dilating to asphyxia, acetyl-beta-methyl choline, heat or mechanical injury. Unusual sensitivity to injury necessitated extreme care in the preparation of the vascular field for observation. Injury results from opening the dura with a cautery, removal of a blood clot, contact of the vessels with dissecting instruments, undue exposure of unopened dura or of pial vessels to drying. The signs of injury are: variable irregularity of vessels, local dilatations and constrictions, constrictions at the origin of arterial branches. These may be present at the beginning of an experiment or appear later. Injured portions appear to be maximally dilated and relatively unresponsive to constrictor or dilator influences. Local dilatations suggestive of injury may appear in response to a rise in blood pressure and later disappear.

Deepening of morphine or of morphine-urethane narcosis has no appreciable effect on the diameter of the rabbits' pial arteries until the depression of respiration becomes sufficient to produce asphyxial dilatation.

Rhythmic changes in pial artery diameter, apparently spontaneous, varying in period and amount, are of frequent occurrence, may appear or disappear during an experiment, may last during an entire period of observation (in one case for 30 hrs.), and often mask the effects of procedures designed to alter pial artery diameter. They are not due to injury.

Effects of section or stimulation of the cervical sympathetic were practically indeterminate, being masked by spontaneous changes in arterial diameter. Stimulation of the cervical sympathetic during asphyxia was ineffective in overcoming the asphyxial dilatation of pial arteries.

Effect of nicotine on oxidations in the brain. H. E. HIMWICH and J. F. FAZIKAS (by invitation). Yale University, New Haven.

The respiratory quotient of unity of the brain indicates the combustion of carbohydrate but yields no evidence as to the form in which that food-stuff is oxidized, for both lactic acid and glucose possess respiratory

quotients of one. Respiratory studies of minced brain made with the aid of the technique developed by Warburg disclosed that nicotine in a concentration of 0.014 M exerts a definite inhibitory action on the oxygen consumption of the brain. In an effort to decide whether the inhibition is specific for various substrates we determined the effect of nicotine on the oxidation of glucose, lactic, pyruvic, fumaric, and malic acids.

The increased oxygen uptake of the brain in the presence of glucose is not influenced by nicotine (+ 3 per cent). The oxidation of lactic acid, on the other hand, is markedly inhibited (-60 per cent). The oxygen uptake caused by pyruvic acid, the first stage in the oxidation of lactic acid, is not inhibited by nicotine. In fact, there is some stimulation (+15 per cent). It is therefore probable that the inhibition of lactic acid oxidation is due to the action of nicotine on the dehydrogenation of lactic acid. In a similar fashion, the dehydrogenation of succinic acid is inhibited by nicotine, for here too the oxygen uptake is diminished (-41 per cent). Fumaric and malic acids did not stimulate respiration. Thus it seems that even in the absence of nicotine, succinic acid may not be oxidized beyond the stage of fumaric or malic acids. Further evidence that the oxidation of succinic acid is incomplete is found in the respiratory quotient of the brain which is 0.57 with this substrate, much lower than the theoretical of 1.14.

These results suggest that glucose may be oxidized by the brain directly without previous glycolysis. They do not however preclude the oxidation of lactic acid in the absence of nicotine. Nicotine may be used as an agent for the differentiation between oxidation of glucose and lactic acid.

Respiratory metabolism and the interconversion of foodstuffs during pupation of the blow fly. F. A. HITCHCOCK and J. G. HAUB (by invitation).

The Ohio State University, Columbus.

Studies have been made on the gaseous exchanges of the blow fly from the time the larvae leave their food until the adults emerge from the pupal cases. The results show that this organism gives off NH_3 as well as CO_2 . By measuring the amount of NH_3 excreted in a gaseous form we have been able to estimate the protein metabolism and calculate the non-protein R.Q. The oxygen consumption of the larvae after they have ceased feeding is about 3.5 cc. per minute per 100 grams. At the beginning of pupation this figure drops until it reaches a low figure of about 0.65 cc. per minute, about the middle of pupation. There is then a rise until at emergence the oxygen consumption is about 1.7 cc. per minute per 100 grams. The protein metabolism as judged by the excretion of NH_3 is quite high during the formation of the pupal cases. It drops off rapidly until by the end of pupation it has reached an extremely low figure. It rises slightly with the emergence of the adult.

The R.Q. of the larvae just before the onset of pupation is about 0.74. During the first half of pupation it fluctuates between 0.72 and 0.74, but during the last half of pupation the quotient definitely falls below 0.70. Upon the emergence of the adult flies the quotient rises to 0.75 or higher.

Chemical determinations of the carbohydrate, fat and protein content at various stages of development show an almost constant percentage of protein (about 20 per cent); a continuous drop in the fat content, which is about 9.2 per cent in the larvae and 7.5 per cent in the adult; and a carbohydrate content which decreases during the first half of pupation and then

increases until at emergence the adult fly has in its body about twice as much carbohydrate as the larva had when it finished feeding.

Since this increase in carbohydrate content occurs at a time when the R.Q. is below 0.70 and when fat is disappearing, it seems likely that fat is being changed to carbohydrate.

A humoral mechanism for peripheral sensory inhibition (adaptation).
HUDSON HOAGLAND. Clark University.

Frequencies of nerve impulse potentials recorded from single axons which branch and form tactile free-ending receptors in frog's skin have been used to study adaptation (failure in frequency of the impulses) in response to pressure. Adaptation to constant pressure is very rapid, but to an interrupted air jet it may be slow and is a quantitatively determinable function of properties of the interrupted jet. Adaptation is not produced by the *activity* of the ending itself since electrically initiated antidromic impulses backfired into the endings are without effect in hastening adaptation to an intermittent air stimulus applied immediately afterward. Gradual application of a non-interrupted air jet which sets up no impulses nevertheless inhibits the endings and renders them unresponsive to an immediately subsequent, normally adequate, interrupted jet. Intermittent air jet stimulation to a region of the skin several millimeters distant from a responsive ending also inhibits the ending to a normally adequate intermittent stimulus applied immediately afterward. These facts suggest the spread of an inhibitory humor released from cutaneous cells by the stimulus. Constant pressure causes adaptation but no appreciable spread of the inhibition is found. Vibratory movement of the skin by the stimulus is necessary to spread the inhibition for appreciable distances. The time curves of recovery from the adapted state are inconsistent with known properties of *isolated* peripheral nerve, as are also curves describing production of adaptation. Adaptation is greatly hastened by washing the underside of the skin with isotonic KCl. Recovery is greatly hastened by washing with Ringer's solution.

The cutaneous non-nervous cells of frog's skin are rich in potassium. Feng has shown that rupture of these cells produces reversible failure of excitability of the nerve endings. The experimental evidence is consistent with the hypothesis that pressure releases, from non-nervous cutaneous cells, potassium which, by temporarily accumulating around the nerve endings, reduces their excitabilities by raising the amount of potassium (K_o) outside of the axon branches and thus reducing the ratio of potassium in the nerve fibers to that outside (K_iK_o), upon which their excitabilities depend.

Permeability of red cells to organic anions. RUDOLF HÖBER (introduced by M. H. Jacobs). University of Pennsylvania, Department of Physiology.

The selective permeability of dried collodion membranes to inorganic cations seems to be a physical analogy to the selective permeability of the red cells to the inorganic anions, and therefore it has been assumed that the surface membrane of the red cells has a sieve-like structure, the different rates of permeation of ions depending on their different size. To prove this concept, the experiments on red cells as well as on collodion membranes have been extended to the organic anions. The permeability of the

red cells has been studied by measurements of the rate of hemolysis in ammonium salt solutions, the permeability of the collodion membranes by following the membrane potentials after changing the electric charge of the collodion by a basic dyestuff. It appeared 1, that the penetration through both kinds of membranes of the anions of the strong carbonic and oxycarbonic acids like citrate, tartrate, lactate, fumarate is comparable in slowness to sulfate; 2, that the anions of the fatty acids C₂-C₆ exceed distinctly the sulfate ion; 3, that caprylate, nonylate, oleate, abietinate, benzoate and salicylate surpass it by far in speed, and 4, that also the anions of the strong sulfonic acids like benzenesulfonate or naphthalenesulfonate pass more rapidly than sulfate. From these results it follows that the ionic volume cannot be regarded as the main controlling factor in these permeabilities. Apparently, beside volume the hydropolarity is a deciding factor in the case of the sulfonates; in the case of the fatty acids the adsorption affinity and the lipoid solubility, both of which increase with the length of the C-atom chain, in the case of benzoate and salicylate hydropolarity beside lipoid solubility. The permeability of the red cells to the organic anions differs strongly from species to species; this is likely to be due to the different ratio of lipoid and porous areas in the surface of the cells.

Observation on the bilaterality of the carotid sinus reflex in primates. E. C. HOFF and L. H. NAHUM. Laboratory of Physiology, Yale University, New Haven.

Heymans found that in the dog unilateral vagotomy abolishes the cardio-depressor reflex previously obtained by stimulation of the carotid sinus on the same side, and concluded therefore that in this animal the carotid sinus reflex is homolateral. We have shown that in primates (monkeys and chimpanzees) each vagus usually innervates preponderantly the region of the S-A node in contrast to dogs in which the right vagus is distributed principally to the S-A node. Further observations were therefore made in monkeys (*Macaca mulatta*) on the cardio-depressor effect produced by mechanical stimulation of the carotid sinus with only one vagus intact. In each animal the right or left vagus was sectioned aseptically in the neck. Thereafter at intervals of about a week the animals were again anesthetized and the cardio-depressor discharge, elicited by mechanical pressure of the carotid sinus on each side, was recorded electrocardiographically. It was found that during the depth of the anesthetic slowing could not be produced by mechanical stimulation although it was regularly obtained by injecting adrenin intravenously. Evidently the number of end-organs stimulated by mechanical pressure is much less than that excited by a rising blood pressure.

After about 2½ hours when the animal was emerging from anesthesia but not yet sufficiently excitable to increase the adrenin in the blood, pressure over each carotid sinus resulted in slowing of the heart whether the right or left vagus was intact. This indicates that in monkeys the carotid sinus reflex is bilateral and that afferent fibers from each carotid sinus affect the center as a whole. The depressor effects obtained were limited almost always to the pace-maker, which suggests that the fibers of lowest threshold in each vagus innervate the pacemaker. Finally, the vagus nerve constitutes the principal efferent pathway of the reflex inasmuch as the slowing of the heart varied from 50 to 100 per cent—much more than could be accounted for simply by reflex inhibition of accelerators (Allen).

Further studies on amino acids in development. VIII. On the physiological value of the amino acids of glutathione and of some proteins in amphibian development. O. HOFFMAN (by invitation) and F. GUERNATSCH. New York University, Washington Square College, New York City. (Read by title.)

In previous studies glutamic acid had occasionally shown a growth stimulating effect. To test it further Rana elamatans tadpoles were fed a non-nitrogenous basal diet and, respectively, glutamic acid, + glycine, + cysteine, + cysteine + glycine, + tryptophane. Glycine + cysteine also were given. Glutamic acid + cysteine or + tryptophane failed early. Glutamic acid + glycine showed fair maintenance but not much growth; glycine + cysteine poor maintenance but comparatively good growth; glutamic acid alone early good, later fair maintenance and comparatively good growth. Most favorable, in general, was glutamic acid + cysteine + glycine, maintenance being very good, growth comparatively good and general appearance above that of the other groups. A mixture of these three acids, the components of glutathione, was definitely favorable.

Other groups of these animals were fed, respectively, gelatin, + tyrosine, + tryptophane, + tyrosine + tryptophane; casein, casein acid hydrolysate + and - tryptophane; casein enzyme hydrolysate + and - tyrosine.

Of the gelatin groups, in respect to growth, gelatin + tyrosine + tryptophane was superior. In maintenance gelatin + tyrosine + tryptophane and gelatin + tryptophane were superior. Both in growth and maintenance all were superior to gelatin alone.

In size and maintenance casein was superior to all gelatin groups. Neither did the casein hydrolysates equal casein. The enzymic hydrolysate was more favorable than the acid hydrolysate from which the humin was not removed and which seemed more toxic. Tyrosine improved the value of the former. Tryptophane did not improve the latter.

On Rana sphenocephala both casein and gelatin showed good developmental value, casein being far superior though the animals did not quite complete metamorphosis.

A study of the influence of polyneuritis on the head-nystagmus of pigeons. O. LEONARD HUDDLESTON and DONALD L. ROSE (introduced by Maurice H. Rees). Department of Physiology and Pharmacology, University of Colorado School of Medicine, Denver, Colorado.

Head-nystagmus of adult pigeons was recorded every other day for a period of one to two months. One group was placed on a diet of polished rice and a control group received mixed grain. Except for the last two or three weeks in which each pigeon was rotated in the light and in the dark on the same day, all nystagmus tracings of this series were recorded in the light. Records of the control and experimental pigeons corresponded very closely in practically every detail until shortly before the onset of terminal symptoms. The outstanding changes noted were a reduction in the nystagmus frequency of rotation-nystagmus, and a complete loss of after-nystagmus. In another series which was rotated in the dark only, and at weekly intervals, the after-nystagmus was not lost until a few hours before the development of other significant changes.

Just prior to the appearance of severe neuromuscular symptoms, decided changes in the head-nystagmus developed. There was a reduction

in amplitude and frequency of the nystagmus, a reduction in the magnitude of the compensatory deviation of the head, irregularities and reversals in the direction of the compensatory deviation, and finally, a complete loss of head-nystagmus. The labyrinthine reflex disappeared before optical-nystagmus was lost and the survival period of such pigeons was not longer than 36 hours.

Ingestion of 6 to 9 grains of powdered yeast, or an injection of an extract prepared from rice polishings into polyneuritic pigeons showing a complete loss of head-nystagmus caused a partial return of rotation-nystagmus in two to four hours. Within twelve to twenty-four hours, the after-nystagmus (if not previously destroyed by habituation) reappeared and the rotation-nystagmus and optical-nystagmus returned to the plateau level.

Because of the very rapid return of the labyrinthine and optical reflexes following the administration of the antineuritic vitamin, it is very suggestive that the neuromuscular symptoms of polyneuritis are caused by a metabolic disturbance of the nervous system rather than by degenerative changes.

Temperature of bone marrow in rabbits. C. B. HUGGINS and B. H. BLOCKSON, JR. (by invitation). University of Chicago.

The problem of the function of the bone marrow, especially the reasons for its location in the bones after birth in healthy mammals and the topographical distribution of red and yellow marrow made it necessary to know the temperature conditions in this organ. In the bones of the extremities of rabbits, under local anesthesia, the cortex was punctured and no. 34 gauge copper-constantan wire thermocouples were inserted and the temperature of the marrow determined by a D'Arsonval galvanometer. Certain precautions must be taken to obtain accurate readings. A definite temperature gradient in the marrow was found, running from the shoulder to the wrist and from the hip to the phalanges of the toes, with the proximal areas hotter, resembling the gradient in the other soft tissue of the extremity. Cooling due to quiet exposure to room temperature (20-26°C.) occurred in the distal areas to a much greater extent than in the proximal. The marrow of the diploe of the skull and sternum resembles the proximal areas. It may be concluded that the bone salts in living bone provide a poor heat insulation.

Inhibition in internuncial neurons. JOSEPH HUGHES (by invitation), G. P. McCOUCH, W. J. SNAPE (by invitation) and W. B. STEWART (by invitation). Pennsylvania Hospital for Nervous and Mental Diseases and Department of Physiology, University of Pennsylvania Medical School.

Gasser and Graham described the cord potential following a single break shock to a dorsal root as consisting of a spike followed by slow negative waves often succeeded by a prolonged positive wave. Hughes and Gasser have shown that on synchronous stimulation of two adjacent dorsal roots the initial spike of the combined cord potential equals the sum of the spikes of the two potentials recorded alone, but that the later components all undergo occlusion. When synchronised with an antidromic volley, however, the result was the algebraic sum of the reflex and antidromic potentials. They concluded that the spike is the potential of afferent fibers and that the subsequent components arise in internuncial neurons.

The present work concerns the effect upon the cord potential of a preceding volley from a contralateral dorsal root in the acute spinal cat. At intervals showing inhibition in the isometric myogram of ipsilateral tibialis anticus there is a reduction in the first slow negative component of the cord potential approximating the percentage of contraction inhibited. Accepting the validity of Hughes and Gasser's conclusion, our result is interpreted as evidence that much of the inhibition occurs in internuncial neurons. It may be significant that the intervals associated with inhibition are those in which the reduced second potential is superposed upon the prolonged positive wave of the first, a relation similar to that obtaining in Hughes and Gasser's cases of inhibition by a preceding ipsilateral volley.

An automatic stimulator. I. F. HUMMON, JR. (introduced by T. E. Boyd.) Loyola University School of Medicine, Chicago. (Demonstration.)

This is a stimulator using amplified current from a photoelectric cell, with rotating disc interrupter. It is similar in principle to the one described by Nicolai (Pflüger's Arch. **225**: 567, 1930) but has certain modifications permitting variations in shock duration, short measurable periods of stimulation, and chronaxie measurements.

Effect of carbon monoxide on the recovery of frog skeletal muscle. J. B. HURSH (introduced by W. O. Fenn). Department of Physiology, School of Medicine and Dentistry, The University of Rochester.

Fenn (1932) found that the respiration of frog muscle increased 1½- to 3-fold when CO was substituted for the nitrogen of the air, and that this increase was due to a burning of CO to CO₂ by the muscle. It was of interest to find out whether the performance of this additional function by the respiratory enzyme system of the muscle would have any effect on the oxidative recovery processes following stimulation of the muscle.

Respirometer experiments in which the gas surrounding of the muscle was alternated from air to the 21 per cent oxygen - 79 per cent CO mixture showed that the excess oxygen consumption of recovery was 52 per cent less for the muscle in CO than for a normal muscle for the same amount of tension developed. Both tetani and twitches were investigated.

Myothermic experiments showed that, corresponding to the diminished oxygen consumption, the delayed heat for the CO muscle amounted to only 47 per cent of the normal delayed heat in air. Corresponding to the findings of Fenn (1932) it was confirmed that CO had no effect on the initial heat or the development of tension in the contraction.

The effect of CO persisted unchanged after the muscle had been soaked in 1/25,000 dilution of sodium iodoacetate in Ringer's fluid, the ratio of total heat over initial heat being, for the air muscle, 1.98; and for the CO muscle, 1.42, showing again a diminution in the recovery heat of 57 per cent.

Thinking that incomplete or greatly retarded recovery might explain the results, lactic acid analyses were performed with the surprising result that no differences in lactic acid concentration could be found after equal periods of recovery in the two gas media.

Until evidence is found of the accumulation of some other metabolic product, it is tentatively concluded that CO increases the efficiency of recovery in frog muscle.

Respiratory characteristics of the blood of the seal. LAURENCE IRVING.
Department of Physiology, University of Toronto.

The common or harbor seal (*Phoca vitulina*) is an active swimmer and diver which shows fine adaptation to a habitat unusual for mammals. In its ordinary respiration the seal successfully encounters many difficulties which the terrestrial mammal could not survive. If there is any adaptation of mammalian blood to habit, the adaptation should appear in the seal.

Blood was examined from 11 seals, about five months old and twenty to thirty kilos in weight. The blood was secured by heart puncture either with, or in a few cases, without anesthesia.

The average O_2 capacity was 29.3 volumes per cent, a value which is distinctly large for mammalian blood. The corpuscles comprised 48 per cent of the blood, and so contained the large quantity of 61.3 volumes per cent of O_2 .

Data were obtained for O_2 dissociation curves of the blood of three seals. At 40 mm. pressure of CO_2 , 50 per cent saturation with O_2 occurred near 30 mm. pressure of O_2 . At lower pressures of CO_2 the O_2 capacity was greater for each pressure of O_2 . In these respects seals' blood is identical with the blood of other mammals.

CO_2 dissociation curves were constructed for the blood of five seals. The average of four indicated about 46 volumes per cent CO_2 at 40 mm. pressure in oxygenated blood. The slope and height of the CO_2 dissociation curve are similar in the seal and many terrestrial mammals.

In spite of the adaptive modifications of seals to their habitat, the system present in the blood and responsible for transport of O_2 and CO_2 is identical with the mammalian type.

Isolation and standardization of a chlorokinetic depressor substance in extracts of mammalian kidney. BENJAMIN JABLONS (introduced by Otis M. Cope). Laboratory of the Jewish Memorial Hospital, and the Department of Physiology and Physiological Chemistry of the New York Homeopathic Medical College and Flower Hospital.

All of the extracts obtained from mammalian kidneys have heretofore failed to show the absence of choline, histamine, adenylic acid or diguanidin. Since many of these bodies produce a depressor effect, it is necessary to obtain a kidney extract which will be free of these substances for it to be specific. Such an extract has been obtained by a process of acid alcohol extraction with neutralization with ammonium salts and fractioning with varying concentrations of alcohol. A crystalline substance crucial in form with arborescent markings can be obtained by repeated extraction with alcohol which is exceedingly soluble in water, and which does not react with Kraut's reagent. It is non-dialyzable and when injected into rabbits or humans, produces a definite drop in the chloride concentration in the blood stream. Increasing amounts of this substance produce a proportionate drop, and this effect has been used for standardization. It prevents the blood pressure raising effects of adrenalin and pituitrin. In rabbits the depression sometimes is equivalent to 100 mm. of mercury. It also produces a drop in the cholesterol content in rabbits and humans of close to 15 per cent of the control figures. This material lowers the blood pressure in human beings and produces a definite increase in the chloride output in the urine. It produces a vaso-dilatation as evidenced by the

increase in peripheral temperature of patients and animals. It produces a marked vasodilatation locally in the rabbit's ear, and the human skin, and at a distance from the site of injection. It increases respiratory metabolism of humans approximately 25 per cent within the first two hours following its injection. It does not give the Sagakuchi reaction. It is not toxic to small animals since 6 per cent fails to kill mice within 24 hours. It is not destroyed by histaminase after incubation for 48 hours. The hypochloremic substance lowers the tonus of the surviving guinea pig's intestinal strip, and fails to contract the virgin rabbit's uterus.

We have also isolated from kidney extract a fraction which interferes with this chloride lowering substance, which when injected into rabbits increases the blood chloride content.

Variations in muscular "tonus." EDMUND JACOBSON. The Physiological Laboratory of the University of Chicago.

The traditional definition that tonus is the slight contraction present in intact or healthy muscle even during resting states is founded chiefly on the interpretation of postures and other gross phenomena, rather than on direct measurement. Extremely sensitive voltage testing equipment now makes it possible to go into this question directly in terms of action-potentials. Apparatus is available capable of detecting transient voltages to within a major fraction of a microvolt, in frequencies higher than 30 per second; but to a considerably higher microvoltage in lower frequencies. The results in numerous measurements in striped muscle of man, the dog and the frog fail to sustain the traditional view. During quiet states in these organisms practically no vestige of tension is detected for periods of minutes at a time. From an energy standpoint it is obvious that if voltages are so extremely slight as to be less than the errors in these methods of measurement, contraction is doubtless physiologically negligible—if not absent altogether. On the other hand, when the resting individual or animal appears grossly quiet, marked action-potentials in various muscles often disclose that the inactivity is far from complete.

According to the traditional view, tonus in striped muscle favors speed of response to stimuli. Tests on reaction times in man disclose an increased speed if there is preliminary slight contraction rather than complete relaxation in the reacting muscle, which accords with the traditional view; but this increase is strikingly small: the completely relaxed muscle can nevertheless respond promptly.

The use of the term tonus in the traditional sense for striped muscle does not seem warranted. It is suggested that tonus in striped muscle be redefined as meaning a state of slight contraction, more or less constant or irregular, often present but sometimes absent in health.

Results of some measurements in smooth and cardiac muscle during moments of relative rest are also discussed.

The innervation of the coronary vessels. KENNETH JOCHIM, ANNE BOHNING, WILLIAM ROTTESMAN and ALEXANDER GRALNICK (introduced by Louis N. Katz). Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, Chicago.

We (Katz, Jochim and Bohning) have shown that the dynamic conditions under which the heart beats have an appreciable effect on the coronary flow. This makes it difficult to judge in the beating heart whether a

given change in flow is due to a chemical or nervous action on the coronary vessels or to this extravascular effect. Some of the contradictory results on coronary innervation obtained in the past may be due to the presence of this extravascular factor. We have recently used a preparation in which the innervated coronary vessels and the heart are perfused with defibrinated blood under constant pressure; the extravascular effect of the beating heart was eliminated by throwing the ventricles into permanent fibrillation. We found in this preparation that: 1, sectioning the vagi caused a decrease in coronary flow; 2, stimulation of the peripheral ends of the cut vagi caused an increase in coronary flow; 3, complete denervation of the heart, some time after sectioning the vagi, caused a persistent acceleration in coronary flow; following ergotamine, this caused a decreased flow; 4, stimulation of the stellate ganglia after section of the vagi caused, on occasion, an increased coronary flow, on occasion a decreased flow. In some experiments these procedures had no effect, but the reverse of the effects described was never seen.

These results strongly suggest that the vagi are coronary vaso-dilators, that the sympathetics contain both coronary vaso-constrictors and vaso-dilators, and that these nerves carry tonic impulses.

Effect of intravenous injection of sucrose on the total osmotic pressure of blood and urine of the dog. A. F. JOHNSON (by invitation), S. F. SEELEY (by invitation), EDWARD J. BALDES and HIRAM E. ESSEX. Division of Biophysical Research and Institute of Experimental Medicine, The Mayo Foundation and The Mayo Clinic, Rochester, Minnesota.

Data will be presented on the study of the total osmotic pressure of blood and urine of dogs, following the injection of 8 grams of sucrose per kilogram of body weight. Measurements are made on samples taken before the injection, during the injection and diuresis, and for a period of several hours following the diuresis. Comparisons will be given of the NaCl equivalent of the sucrose injected and the NaCl equivalent of the urine during diuresis. The determinations of osmotic pressure are made by the "drop" mieromethod of Baldes.

Influence of calcium salts and calcium salts in combination with parathyroid extract upon blood sugar. I. Calcium gluconate. JOSEPH L. JOHNSON.

Department of Physiology, Howard University, Washington, D. C.

Calcium gluconate alone and in combination with parathyroid extract was administered to dogs under ether anesthesia and blood sugar changes noted. A saturated solution of calcium gluconate was introduced into the jejunum at intervals of from 30 to 45 minutes. When calcium gluconate was administered in combination with parathyroid extract the extract was given intravenously. Eight experiments were conducted two of which were controls. Blood was drawn from the portal vein at thirty minute intervals and determinations for blood sugar, serum calcium and serum inorganic phosphorus were made. Following the administration of calcium gluconate and of calcium gluconate in combination with parathyroid extract the portal blood sugar fell rapidly and continuously during the period of the experiment. The lowest levels were reached under the influence of the combined action of calcium gluconate and parathyroid extract. In the control animals the portal blood sugar showed a con-

sistent rise, as might be expected under the influence of ether anesthesia. From these experiments it is concluded that the fall of blood sugar is due to impaired absorption of glucose from the gastro-intestinal tract.

Quantitative determination of blood urea by use of the magneto-optic method.

HERMAN D. JONES and RAY EVERE (introduced by William deB. MacNider). Laboratory of Organic and Biological Chemistry of the Alabama Polytechnic Institute, Auburn, Alabama.

The minima for urea were located on the magneto-optic scale. These were found to be at 19.82 and 19.90. After the positions were located the sensitivity of the apparatus was determined as described by Allison and from this constant factor quantitative determinations were possible. Quantitative analysis of unknown urea solutions were first made as a check against our technique and ability to do quantitative work, with the following results:

- 1st. Solution present 2.5×10^{-4} grams, found 1.9×10^{-4} grams
- 2nd. Solution present 2.9×10^{-4} grams, found 2.75×10^{-4} grams
- 3rd. Solution present 3.07×10^{-4} grams, found 2.76×10^{-4} grams
- 4th. Solution present 2.34×10^{-3} grams, found 2.53×10^{-3} grams
- 5th. Solution present 2.47×10^{-4} grams, found 2.55×10^{-4} grams

As a check on quantitative blood analysis each sample of blood was run by both the magneto-optic and by the Van Slyke and Cullen modification of Marshall's urease method. The chemical analyses were made by one worker while the observers were making the determinations with the magneto-optic method and the results reported to a third party. The following are representative of the results obtained:

	<i>Van Slyke & Cullen Method</i>	<i>Magneto-optic Method</i>
Blood no. 1.....	11.14 mgm.	12.80 mgm.
Blood no. 2.....	18.25 mgm.	19.15 mgm.
Blood no. 3.....	17.74 mgm.	18.22 mgm.

A number of bloods have been checked in this manner with the results agreeing always as given. One-tenth cubic centimeter of blood was used in making the analyses and the calculations were made as follows,

$$\frac{4.7}{1.4} \times \frac{10^8}{10^{12}} = \% \text{ urea}$$

4.7×10^{-12} is the sensitivity constant for urea and 1.4×10^{-8} is the concentration of the urea in the blood.

By use of the magneto-optic apparatus it has been possible to detect two minima for urea, 19.82 and 19.90 due to H^2 and H^1 respectively. The sensitivity of the Apparatus for urea for the two observers in this work is 4.7×10^{-12} . No minima were found in pure pyrex distilled water at these two positions, but were found in blood and urine. The magneto-optic method of analysis can be used for quantitative determinations of urea in blood and urine.

Blanks were run on every cell and all beakers used in making the dilutions in order to preclude any possibility whatsoever of any misleading results.

The effect of lipoids including cholesterol on the rate of evaporation of water from the skin. K. K. JONES and D. E. MURRAY (introduced by A. C. Ivy). Department of Physiology, Northwestern University Medical School, Chicago, Illinois.

Since the sebaceous secretions are lipoidal in nature and contain cholesterol, this study was undertaken to determine the effect of removal of lipoids from the skin and replaced by petrolatum and petrolatum containing cholesterol in various concentrations on the "insensible loss" of water from the skin, the water that is lost at temperatures below 30°C.

An apparatus was designed to measure accurately the amount of water lost from a given area of skin, the middle finger of the left hand being selected as the best to use.

The time and flow of dry air was constant in all tests and the effect of temperature between 20° and 30°C. was measured. The results are best shown by plotting the loss of water against the temperature, but the chief findings may be summarized in the following statements.

The normal epidermis loses water at a faster rate than when it is deprived of its lipid constituents by ether washing. A film of petrolatum base protects the skin from the loss of water and reduces this loss to one-half that lost by the normal skin.

The addition of 2 per cent cholesterol and 10 per cent cholesterol to this base increases in both cases the loss by one-half the difference between that of normal skin and that of the petrolatum protected skin. Five per cent cholesterol in petrolatum does not increase the loss to any extent over that of the petrolatum base.

Electrical stimulation of the hypothalamus in the waking animal. H. KABAT, B. J. ANSON and H. W. MAGOUN (introduced by S. W. Ranson). Institute of Neurology, Northwestern University Medical School.

By cementing the electrode in place after it had been oriented with the Horsley-Clarke instrument, it was possible to stimulate the brain of the unanesthetized animal. Stimulation of the hypothalamus resulted in pupillary dilatation, increased rate and amplitude of respiration, erection of hair, inhibition of gastro-intestinal peristalsis and frequently clawing, struggling and urination. The cat appeared to be enraged. In contrast, during stimulation of the anterior commissure, internal capsule, infundibulum or thalamus with the same strength of current, the animal showed no evidence of sympathetic discharge or of emotion.

Increased peristalsis never resulted from stimulation of any of these regions. Stimulation of the anterior commissure frequently caused urination and rhythmic crying. Rhythmic stepping and mastication were obtained from the internal capsule. Very strong excitation of the hypothalamus, internal capsule or thalamus caused convulsions, those obtained from the thalamus persisting for 1 to 2 minutes after stimulation.

The response of a cat to a barking dog, as described by Cannon, is very similar to its response to weak stimulation of the hypothalamus. Bard showed that sham rage occurred after ablation of all of the brain rostral to the hypothalamus but disappeared when the hypothalamus was removed. We believe, therefore, that the hypothalamus is the integrating center for the reactions of strong emotion.

The excretion of inulin, creatinine, xylose and urea in the rabbit. BERNARD I. KAPLAN (by invitation) and HOMER W. SMITH. Department of Physiology, New York University, University and Bellevue Hospital Medical College.

The rabbit has in our experience proved to be a difficult animal in which to maintain a relatively high rate of urine formation. The administration of water *per os* in large quantities is likely to lead paradoxically to a low urine flow, and not infrequently to convulsions and death. This result might possibly be due to water intoxication.

Using rabbits of 2.0 to 4.0 kgm., and such quantities of water as produce urine flow up to 5 cc. per sq. m. S.A. per minute, we have examined the simultaneous clearances of inulin, creatinine and xylose, and in other experiments the simultaneous clearances of xylose and urea.

The ratio of the simultaneous inulin and creatinine clearances in a series of 44 observations averages 1.0 with a variation of about 5.0 per cent. This ratio is maintained at levels of plasma creatinine ranging from 6 to 125 mgm. per cent, and at U/P ratios of inulin ranging from 10 to 125 (i.e., urine flows from above 5 cc. to 0.1 cc. per sq. m. S. per minute). In a smaller series of observations the xylose/inulin ratio varies between 0.5 and 0.6. This ratio appears to depend on the rate of urine flow, the xylose clearance increasing relative to the inulin clearance at higher rates. The urea clearance in a large series of observations is invariably below the simultaneous xylose clearance and averages not more than 60 per cent of the latter when the rate of urine flow is 5.0 cc. per sq. m. per minute.

Under the conditions of these experiments the clearances of all substances are markedly reduced at urine flows below 1.0 cc. per sq. m. per minute. Since inulin and creatinine behave alike in this respect, it would appear that, in addition to reabsorption of urea (and perhaps xylose), there is a marked reduction in glomerular activity at low urine flows. Failure to appreciate the influence of the effect of rate of urine flow upon the excretion of these substances has led to misinterpretation of renal function in this species.

The chemistry of the contents of the small intestine: The fasting contents, reaction and osmotic pressure after acid, bicarbonate, water and glucose feeding. W. G. KARR and W. O. ABBOTT (by invitation). Gastro-Intestinal Section of the Medical Clinic, University Hospital, Philadelphia, Pa.

By use of the Miller and Abbott¹ two lumen intestinal tube it is possible to intubate the whole length of the small intestine in a relatively short time. Fasting samples of fluid were obtained at various levels in the small intestine and analysed for pH, osmotic pressure ($\text{Cl} + \text{HCO}_3$), solids, Ca, P, Tot.N., non-protein N., as a basis for further work. The pH varied from pH 2.64 to pH 7.80, over 50 per cent being between 6.5 and 7.5. Osmotic pressure varied from 138 milliosmoles to approximately 300 which is isotonic with the blood plasma.

The pH and osmotic pressure relationships were studied at various levels after the oral administration of HCl and NaHCO₃ in varying concentrations.

¹ Miller, T. G. and W. O. Abbott. Am. J. Med. Sci. 187: 595, 1934.

Similarly the osmotic pressure was studied after water and hypertonic glucose ingestion. Water induced a hypotonic state for an indefinite period of time. After hypertonic glucose the intestinal contents may be in osmotic equilibrium with the blood plasma or in some cases they may be definitely hypertonic.

In general it may be said that the reaction (pH) is controlled almost entirely by the rate of passage of the contents through the stomach and small intestine in relation to the rate of secretion of the alimentary canal. The small intestine also makes a sluggish attempt to keep its contents at all times in osmotic equilibrium with the blood plasma.

The variability in the distribution of the outflow from each of the three major coronary arteries. LOUIS N. KATZ, WILLIAM WEINSTEIN (by invitation) and KENNETH JOCHIM (by invitation). Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, Chicago.

In the course of experiments dealing with denervated coronary vessels perfused under constant pressure and in which the ventricles were fibrillating, we have had occasion to compare the coronary inflow with the total coronary outflow. Within the experimental error, the two flows were equal at any given time. However, the distribution of the coronary outflow between the coronary sinus, the thebesian vessels of the right heart and those of the left heart varied considerably in different experiments. It was not possible to establish an average value for the distribution of coronary outflow between these paths.

In a number of experiments we determined the partition of outflow for each of the three major coronary arteries by temporarily clamping the other two vessels. A wide variation in partition of outflow from each artery occurred in the different experiments. However, it was found that the right circumflex coronary artery emptied practically entirely through the right thebesian vessels, the left circumflex artery emptied mainly by way of these same vessels, and the left descending emptied equally between the right thebesian vessels and the coronary sinus. The thebesian vessels of the left ventricle constituted a very small portion of the channels through which coronary blood escaped. A wide variation was also found in the proportion of the total coronary flow carried by each of the three coronary arteries.

Results such as these lend support to the view that measurements of flow from the coronary sinus cannot be used as an accurate index of the total coronary blood flow.

Protection by peripheral nerve section of the gastro-intestinal tract from ulceration following hypothalamic lesions. A. D. KELLER. Department of Physiology and Pharmacology, University of Alabama.

Ulceration encountered in the stomach and duodenum following hypothalamic lesions and hypophysectomy has been essentially of two types, namely: 1, hyperemia and hemorrhage of the body of the stomach and proximal duodenum with resultant bleeding into the lumen, and 2, characteristic crater formation in the body and pylorus of the stomach and proximal duodenum.

Although the changes definitely occurred antemortem, the question arose as to whether they were 1, simply an indirect result of the generalized altered physiological condition resulting from the central lesion, or 2, a

matter of direct hyperactivation (irritation or release) of central groups of cells having synaptic connections with the outflow of fibers supplying the gut. Again the fact that when the hemorrhagic states were encountered, craters were not evident and vice versa, raised the question as to whether the two end results observed were 1, different stages of the same process, or 2, two separate and distinct mechanisms.

In an attempt to answer these questions, bilateral vagotomy in one group and bilateral abdominal sympathetic avulsion in another was performed previous to placing anterior hypothalamic lesions. No gross changes occurred in 14 of 25 vagotomized dogs, while typical hemorrhagic states were encountered in the other 11. There was no evidence of crater formation in this group. Normal guts were encountered in 16 of the 33 sympathectomized dogs; while crater formation occurred in the remaining 17. In this group no hemorrhagic states were encountered. Thus, vagotomy does not protect the gut from the hemorrhagic states and sympathectomy does not protect from crater formation; while the opposite protection is strongly indicated though not conclusively demonstrated.

It is also indicated that two separate mechanisms were activated centrally, one acting via the vagi, the other via the sympathetic. Certainly from the gross and histological changes the peripheral site of increased gastric activity in both instances was in the mucosa of the body. The particular components of the gastric juice that was affected in each instance is indicated in our present results; however, we propose to perform other experiments for verification.

This work was aided by a grant from the Committee on Scientific Research of the American Medical Association.

A cat and a dog exhibiting unilateral overflexion. A. D. KELLER. Department of Physiology and Pharmacology, University of Alabama. (Demonstration.)

Contralateral overflexion of the limbs following hemisection of the brain stem at a pons level has been previously described. (Proc. Soc. Exp. Biol. and Med. **29**: 189, 1932; Proc. of this Society, 1933.)

The brain stem of the cat to be exhibited was hemisected on the right side in the pons in July, 1934. Its present overflexion of the left limbs has persisted constantly since operation. It is to be noted that in ordinary walking the foreleg is raised high off the floor by the moderate flexing of all joints, also that following the flexing of the rear leg it is thrust backward characteristically before the foot is again placed on the floor. At times it will be noted that the foreleg is maximally flexed thus pulling the foot up against the ear on that side.

The dog to be exhibited had in July, 1934, a longitudinal lesion placed in the pons somewhat lateral to the midline. Following the operation the dog was markedly incapacitated. In September, however, she was able to get about the yard adequately, though awkwardly, the overflexion of the right legs being prominent. In November, the dog was subjected to an epileptic seizure of unknown origin. Directly following overflexion became spontaneous and has persisted. Does not the picture as you see it simulate the chorea-athetoid movements seen clinically which in the past have been explained on the basis of injury to the cerebello-rubro-spinal pathway? That the condition is not explainable on this basis is evident by the fact that a brachium conjunctivum in the monkey can be sectioned

without precipitating dysfunction in feeding movements. Also, more recently the following procedures have been successfully performed without precipitating any type of dysfunction in the dog: 1—Section of a brachium conjunctivum; 2—section of brachium pontis, and 3—complete unilateral removal of the neocerebellar cortex.

In the event these animals do not survive or are not in good health at the time of the meeting, moving pictures of them will be shown.

Motion pictures of animals having various brainstem lesions. A. D. KELLER.

Department of Physiology and Pharmacology, University of Alabama.
(Demonstration.)

A. See the rubrospinal tracts in the monkey, Arch. Neurol. and Psychiat.
32: 1253, 1934.

1. Monkey (10) showing typical cerebellar muscular incoordination and spontaneous tremor.

2. Monkey (6) with unilateral section of left brachium conjunctivum showing:

(a) feeding movements, (b) facial epileptic attack. The feeding movements in the monkey are limited but note the handling of a banana and the cleaning of its fingers with no evidence of ataxia.

3. Symptomless rubrospinalless monkey (no. 12) at various post-operative periods showing feeding and righting movements.

4. Monkey (5) showing typical reflex standing, the essential walking pattern, tonic neck reflexes and the absence of righting reactions.

B. Complete generalized muscular atonia in an unanesthetized cat and dog.

The lesion in each instant being a bilateral transverse lesion in pons involving the medial quarter segments and the medial portion of the lateral quarter segments, the cerebellum and vestibular nuclei being uninjured.

The separation of the heat loss and the heat production mechanisms in chronic preparations. A. D. KELLER. Department of Physiology and Pharmacology, University of Alabama. (Read by title.)

The elimination of the heat maintenance mechanism without affecting the heat loss mechanism has been reported in acute midbrain and hypothalamusless cats. It is evident, however, that acute preparations do not demonstrate that structures essential to heat-maintenance are located at the thalamic level or within the hypothalamus, since it would be possible for them to be located in the cephalic midbrain and be temporarily de-functioned.

The inability of 1, a completely midbrain dog (section caudal to posterior commissure dorsally and the caudal tip of the mammillary bodies ventrally), and 2, an essentially hypothalamusless dog (bilateral severance of the dorsal and caudal hypothalamic connections) to shiver and to maintain their rectal temperatures without incubation on the 28th and 32nd day after operation demonstrates that the maintenance mechanism is actually located in the hypothalamus.

We can consider the above times after operation as meeting the tests of chronicity since dogs with lesions sufficiently extensive in the hypothalamus to eliminate heat regulation temporarily began showing evidence of heat maintenance after several days; recovery was prominent in two weeks, and the steady state was attained in approximately three weeks. Thus

dog 474 showed no evidence of heat maintenance for the first few days. Then it was necessary to progressively lower its environment temperature in order not to overheat it. By the 15th day its temperature was maintained in an unheated cage. She did not shiver until the 10th day, her temperature being lowered to 28°C. on previous occasions in an attempt to induce shivering. On the 33rd day she maintained a normal rectal temperature for 12 hours at 44°C., whereas during the succeeding 12 hours at 26°C., the rectal temperature fell to 31.2°C. in spite of prominent shivering.

Chronic preparations with complete unilateral removal of or with bilateral removal of approximately the ventral $\frac{2}{3}$ of the hypothalamus exhibited no alteration in heat regulation. It was only when the dorsal $\frac{1}{3}$ was encroached upon that a temperature deprivation effect could be demonstrated. It was necessary to reduce this dorsal $\frac{1}{3}$ considerably before a deficiency could be demonstrated other than by severe environmental change as in dog 474.

This work was aided by a grant from the Committee on Scientific Research of the American Medical Association.

Adiposity with normal sex function following extirpation of the posterior lobe of the hypophysis in the dog. A. D. KELLER and WM. NOBLE (by invitation). Department of Physiology and Pharmacology, University of Alabama. (Read by title.)

In the course of studies on carbohydrate metabolism we found occasion during the spring of 1933 to remove the posterior lobe of the hypophysis in five mature female dogs. The immediate and persistent tendency toward adiposity (sleekness and plumpness) which these dogs displayed following operation, in spite of seizures of disease as mange and distempers, was strikingly noticeable. This tendency to adiposity was particularly present in dog 130 and during the summer of 1934 it became even more pronounced. Enhanced appetites were present in varying degrees in these dogs but were particularly pronounced in 130. In September, 1934, in an 8 hour period she drank 5000 cc. milk which ballooned her abdomen as if bloated and before the end of the test undigested milk was passed by rectum. Her water intake during a similar period was less than 25 cc.

All the dogs bred regularly every six months, dog 92 having since operation delivered two litters of 5 pups while 130 delivered a litter of 8.

Dogs 92 and 130 were recently terminated. Serial cell and fiber tract studies revealed that in 130 the pars intermedia and the infundibular process were cleanly removed with no injury to the pars anterior, the pars tuberalis, the infundibulum or the hypothalamus. In dog 92, in addition to removal of the posterior lobe, approximately $\frac{1}{2}$ of the anterior lobe was destroyed; while there was no injury to other structures. The 3rd ventricle was not opened at operation in either instance.

Previous to termination of these animals, we had suspected hypothalamic injuries because of 1, the fact that our chronic dogs with hypothalamic lesions showed the same tendency to adiposity and exhibited the same markedly enhanced appetites, and 2, the recent tendency in experimental literature to divorce the hypophysis from any relation to adiposity and to associate it with hypothalamic injury alone.

The absence of hypothalamic injury as well as the presence of an intact

anterior lobe in 130 forces us to again focus our attention upon the "pars intermedia-infundibular-process" complex as possibly the structure deranged when adiposity follows injuries at the brain base.

This work was aided by a grant from the Committee on Scientific Research of the American Medical Association.

Hypoglycemia following experimental hypothalamic lesions. A. D. KELLER, W.M. NOBLE (by invitation) and P. D. KELLER (by invitation). Department of Physiology and Pharmacology, University of Alabama. (Read by title.)

The blood sugar level has been followed in 90 dogs after placing mechanically various types of lesions in the hypothalamus. The routine bloods taken are indicated in table. Food and fluid were withheld for 48 hours after operation unless pronounced hypoglycemia occurred. It is necessary to evaluate each dog's curve on its preoperative level rather than group averages because of seasonal and local weather variations. July normals ranged from 50 to 80 and January's from 90 to 140. November 20th with environmental temperature 59°F. Dog O₂'s value was 84. November 24th, environmental temperature 35°F., its sugar was 112.

DOG	A.M.		OPERATION DAY		1ST DAY		2ND DAY		3RD DAY	
			Early p.m.	Late p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.
341	66.7		41.1	36.2	87.7		70.4	57	70.4	Dead
				Food						
360	83.4		76.9	80.0	82.0		35.0	64.5	Dead	
369	60.7		52	45.4		53.2	63.3	78.1	83.3	79
										Chronic
389	69.7		33.3	35.7	36.0	Dead				
484	111.1	Operation		112.3	108.0	105.2	99.0	101.0	6th day	—103
485	96.1			68.9	58.8	50.0	Dead			
486	126.1			111.1	142.8	153.8	Dead			

Marked or maintained hyperglycemia (dog 486) was infrequent. Slight hyperglycemia during the anesthesia recovery period often occurred. The maintenance of a constant level (484) occurred frequently. Pronounced hypoglycemia, immediate (341, 389) or delayed (360) was striking. Dog 341 was carried for few days and 369 to chronicity by frequent feeding. Dog 360 recovered from profound shock with convulsions when glucose was administered (35.3–64.5). In no instance was the hypophysis injured in placing lesions.

Falling out of heat regulation, weakness and unresponsiveness (cachexia hypophyseoprvia) are not necessarily evidence of hypoglycemia. These symptoms appear hours antemortem in all dogs dying within two weeks after hypothalamic lesions or hypophysectomy. Hypoglycemia, as determined by blood analysis or recovery after glucose, occurs more frequently after hypophysectomy than following hypothalamic lesions.

This work was aided by a grant from the Committee on Scientific Research of the American Medical Association.

The retinal response of the pigeon eye to lights of various wavelengths. E. H. KEMP (by invitation) and C. H. GRAHAM. Clark University.

Kohlrausch has reported that retinal potentials from the eyes of various animals assume different forms with different wavelengths of stimulating light; and further, the retinal potentials for different wavelengths cannot be made similar no matter how intensity is varied. This observation is of theoretical significance for the question of color vision and its localization, and for this reason we have attempted a verification.

Retinal potentials were registered, by means of a direct-coupled amplifier and a string galvanometer, from the eye of the pigeon, an animal whose color functions are well known. Various wavelengths of light were provided by Wratten monochromatic filters, and intensity was varied by means of neutral tint filters. The energy for each color was measured by means of a thermopile and galvanometer.

The variation in the form of the retinal potential with variation in the intensity of white light is complex. At low intensities the response consists of a positive deflection which reaches a final steady value. As intensity increases, the positive response becomes larger and drops somewhat to the final level. With further increase, the positive response is preceded by a negative deflection; and at the highest intensities the final level becomes negative. These changes are superimposed on a small, slow, positive deflection.

The intensity effect for all wavelengths over a wide range of intensities parallels that found with white light, and it is possible to find responses at various intensity levels for different wavelengths which are similar throughout the spectrum. Too, one can find examples in the white light series of all the responses which Kohlrausch associated with specific wavelength stimulation.

An analysis of the results gives slight evidence for a Purkinje effect with variation in intensity. The visibility maximum for low to medium intensities is at *ca* wavelength 500 m μ .

Culture of mammalian lymph node: comparative effects of autogenous serum marrow extract and saline chick embryo extract. JOSEPH T. KING. Department of Physiology, University of Minnesota.

Previous experience with serum marrow and saline chick embryo extracts had given the writer the impression that the former promoted a more rapid, denser and more regular migration of lymphoid cells from fragments of adult rabbit lymph node.

Cultures were studied in these two media. The embryo extract used was a concentrated extract of 6-day chick embryo in Tyrode made according to the method described earlier by the author. The marrow extract was made according to Maximow with autogenous serum. Care was exercised to assure having fragments of comparable size in each medium. Maximow slide technique was used. Observations were made at 4 hours, 1, 2, 3 and 4 days. Cultures were fixed for *in toto* mounts and serial sections at these intervals.

From this preliminary survey the following tentative conclusions are drawn: Under these experimental conditions the cultures in marrow extract showed more rapid, more extensive, more regular and denser migration of lymphoid cells than those in embryo extract. The cells were not strikingly ameboid.

Cultures in embryo extract showed less extensive and thinner migration. The cells were strikingly ameboid and this condition persisted although there was little widening of the rim of migrated cells after the first 24 hours.

There was also a difference noted in the activity of the polyblast group in these extracts. The proliferation started earlier and was more intense in the marrow extract.

As a coagulant for heparinized plasma, the marrow extract is definitely inferior to embryo extract. The clotting time is relatively long, and the fluid is not well retained.

Further experiments are in progress with a view to determining whether the desirable properties of these extracts can be combined to provide a more suitable medium for the study of lymphocytes during the rather limited period that these cells remain active as such.

Diurnal variation in reaction time and its relation to body temperature.

N. KLEITMAN, S. TITELBAUM (by invitation) and P. FEIVESON (by invitation). University of Chicago.

Simple reaction time to light and sound, and reaction time with choice to colored lights were studied on five male subjects. The reaction time in all cases was high early in the day, decreased to a minimum during the afternoon, and rose during the evening. Simultaneous determinations of the mouth temperature revealed an inverse relationship between changes in body temperature and reaction time, particularly reaction time with choice. The higher the temperature the quicker the response. The speed of response followed pretty closely the diurnal curve of body temperature, in the same manner as did previously reported diurnal variations in performance and in steadiness (This Journal, 104: 449, 1933).

A chemical analysis of the duration of barbiturate action. THEODORE KOPPANYI. Department of Pharmacology of Georgetown University School of Medicine, Washington, D. C.

In previous communications it was shown that barbiturates are distributed almost uniformly in all parts of the central nervous system and that barbital is eliminated from the brain at a slower rate than from other organs. This present study was undertaken to furnish a chemical explanation for the difference between the physiological actions of the so-called long and short acting barbiturates.

Barbital and phenobarbital were chosen as representatives of the long acting, and nembutal of the short acting barbiturates. Besides the difference in their persistence of action, barbital and phenobarbital if injected in anesthetic doses intravenously produced their effects after a longer latent period than nembutal. Both of these phenomena were successfully accounted for by the following experiments: a, animals receiving varying amounts of barbiturates were killed at different brief intervals after administration of the drug, and the organs analysed for their barbiturate content; b, in other instances 100 mgm. of each of the three drugs were administered singly in 100 mgm. doses and the organs analysed 12 hours after injection.

Following nembutal administration the barbiturate content of the brain was higher, following barbital and phenobarbital administration, however, it was lower than that of other organs. (Nembutal: cerebrum

0.120, liver 0.080, kidney 0.080 mgm. per gram. Barbital: cerebrum 0.030, liver 0.067, kidney 0.060 mgm. per gram.)

The second series of experiments revealed that the barbiturate content of the brain of animals receiving barbital and phenobarbital remain high after 12 hours compared to other organs, whereas the nembutal-treated animals showed no detectable amount of barbiturate in the brain or elsewhere.

The barbiturate content of the medulla after barbital or phenobarbital administration is lower than that of the cerebrum, while in the case of nembutal usually the reverse holds true. (Barbital: cerebrum 0.04, medulla 0.026; nembutal, cerebrum 0.058, medulla 0.063 mgm. per gram.)

In conclusion it should be stated that: a, the quick onset of action of nembutal may be attributed to its initial high concentration in the brain; b, its great acute toxicity to its relatively high concentration in the medulla, and c, its short duration of action to its quick disappearance from the central nervous system. The fact that nembutal also disappeared from other organs proves its rapid destruction in the body including the brain.

Studies on the T potential of the tortoise heart. M. R. KRASNO (introduced by J. A. E. Eyster). University of Wisconsin Medical School.

The method of study is the same as that previously applied to the QRS potential. The heart is placed in the center of a circular disc conductor and the potential curves recorded along various axes at different distances from it. Since the potential distribution due to charges near the center of a field of this type is known, it is possible to infer the nature of the electrical changes in the heart from the character of the recorded potential curves.

The T potential deflection shows a progressive change in form from monophasic to diphasic and back, much like the change characterizing the QRS complex. In hearts well filled with blood, the main deflection of the T potential curve is in general oppositely directed to the principal QRS deflection, while the range over which the T deflection is monophasic is considerably less than the corresponding QRS range. As in the analysis of the QRS potentials, the assumption of two electrical dipoles, separated in time and direction, suffices to explain the form of the T potential curves. The angular separation of these dipoles is greater than that of the two QRS dipoles, and no two of the four dipoles accounting for QRST coincide in direction. The potential curves due to the two T dipoles separately may differ considerably in form.

The T deflection is much more variable in amplitude and form than the QRS group, and more readily affected by changes in experimental conditions. Thus in hearts whose blood has been allowed to escape, the T deflection is in general much larger, in the same direction as the main QRS deflection, and monophasic through all but a very short angular range.

The so-called isoelectric period, comprising the ST interval, is the potential summation of the very slow period of growth of the two dipoles, and is therefore truly isoelectric only on the axis for which these potentials are equal and opposite.

The present working hypothesis to account for the T potential is that it consists in part of the reversal of membrane processes responsible for the QRS complex, and in part of potentials of other origin which may greatly modify its form and magnitude. The experiments are being continued.

Effects of prolactin and follicle-stimulating hormone on the adult dove testis.

ERNEST L. LAHR (by invitation), OSCAR RIDDLE and ROBERT W. BATES (by invitation). Station for Experimental Evolution, Carnegie Institution.

Our earlier study of opposite effects of the two hormones on weight of adult testes has been extended, prolactin preparations heated to destroy growth hormone have been used, and a histological study made. Prolactin, injected during 7 to 13 days in daily doses varying from one-sixth unit to 100 units, diminished testis size in proportion to dosage. Highest dosage thus reduced adult testes to about 8 per cent of their original size. Larger doses of previously heated prolactin effected a similarly rapid and extensive reduction of testis size. Probably all elements of the testis share in this remarkable involution. Pituitary F.S.H. was administered during 7 to 10 days in doses of 1 to 10 mgm. daily; a progressive increase in weight, up to 65 per cent above that of the normal adult testes, was obtained. Mare serum, in contrast with prolan, induced similar growth in the adult bird testis. In these supersized testes the germinal elements at least are histologically normal and show very active spermatogenesis.

Reflex pathways concerned in inhibition of hunger contractions by intestinal distension. JOE LALICH (by invitation), W. J. MEEK and R. C. HERRIN.

University of Wisconsin Medical School.

Several workers particularly Brunemeier and Carlson and Thomas have shown that distention of a jejunal Thierry loop in an otherwise intact animal may inhibit gastric motility. The present report deals with the successive elimination of the possible extrinsic pathways concerned in this reflex. Eleven dogs were trained to lie quietly while gastric records were obtained by the balloon method. In seven animals the vagi were sectioned above the diaphragm. Reflex inhibition persisted showing that other pathways than the vagi could function. In four animals vagotomy followed by section of the right and left splanchnics resulted in loss of the reflex. This showed that there were no connections through the lumbar chains and that the reflex, since it had persisted after vagotomy, could be mediated by the splanchnics alone. In three animals removal of both splanchnics and the lumbar chains did not interfere with inhibition. It was therefore proved that the reflex could occur either through the vagi or splanchnics. Necessarily each pathway contained afferents from the intestine and efferents to the stomach. In three animals in which vagotomy was followed by section of the right splanchnics there was one failure of the reflex. In three animals in which there was vagotomy followed by section of the left splanchnics there was one animal in which the response was doubtful. It is concluded that reflex inhibition of hunger contractions by distention of the jejunum may take place either through the vagi or splanchnics, the former pathway probably being more effective.

Some observations regarding the fate of intravenously injected calcium chloride.

A. M. LANDS and P. H. WOODARD (introduced by O. O. Stoland).

Department of Physiology, University of Kansas. (Read by title.)

Following the intravenous injection of a soluble salt of calcium there is a transient rise in the concentration of blood calcium, but the excess rapidly leaves the blood plasma. We have confirmed other workers in showing that it does not pass into the red blood corpuscles nor does excretion play

any significant rôle in this rapid disappearance. To determine the relative importance of the soft tissues and bones on the rate of disappearance from the blood stream, we injected intravenously the following groups of dogs with enough 5 per cent calcium chloride solution to give 38 to 66 mgm. of calcium per 100 cc. of plasma:

1. Anesthetized intact dogs (3 experiments).
2. Anesthetized dogs after ligation of the blood vessels supplying skeletal parts (7 experiments).
3. Starling heart-lung preparations in dogs (3 experiments).

In group 1 the injected calcium excess rapidly leaves the blood stream, 59 to 80 per cent disappearing during the first five minutes and 95 per cent in 180 minutes. In group 2 it was found that ligating the blood vessels to skeletal parts has no effect on the rapid disappearance of calcium from the blood stream immediately following injection but retards the rate of removal throughout the remainder of the experimental period. At the end of 150 to 180 minutes there may be as much as 24 per cent of the injected calcium still circulating. In group 3 essentially the same results are obtained as in group 2.

We believe these results justify the conclusion that immediately following the injection of a soluble calcium salt such as calcium chloride there is a rapid diffusion of calcium into the tissue spaces until some equilibrium is reached with that in the blood stream. Following this rapid diffusion there becomes apparent slower reactions capable of further reducing the excess calcium in the blood stream. These reactions can occur in the absence of skeletal tissues although their rate may be reduced. Further investigation of the nature of these reactions is in progress.

A new method for the investigation of the electrical properties of living tissues.

C. T. LANE (by invitation), W. S. McCULLOCH (by invitation), C. H. PRESCOTT¹ (by invitation) and J. G. DUSSE DE BARENNE. Laboratory of Neurophysiology, Yale University.

A fluctuating voltage is applied to the specimen and a series resistance. The voltage across the specimen produces horizontal deflection, and that across the resistance vertical deflection of the spot on the fluorescent screen of a cathode ray oscilloscope. Balanced amplifiers may be used if desirable.

Since the oscilloscope draws inappreciable current, these simultaneous deflections measure respectively the instantaneous voltages across and currents through the specimen. The resulting luminous figures may be photographed for subsequent analysis.

If the specimen obeys Ohm's law any applied voltage gives a straight line whose slope measures the resistance.

Experience has shown that with non-polarizable electrodes, living tissues, so far as investigated, are approximately linear circuit elements having capacitative reactance. Sinusoidal voltage applied to them gives an ellipse. Impedance and phase shift may be computed from its eccentricity and slope of major axis, or it may be transformed into a straight line at 45° by adjusting the series resistance shunted with appropriate capacity.

Polarizable electrodes with sinusoidal voltages and non-polarizable electrodes with non-sinusoidal voltages produce figures complicated by the

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presence of higher harmonics. Their analysis is difficult, nor are they transformed into straight lines by simple series impedances.

The greatest complexity of harmonic structure with translation and distortion of the figure results when polarizable electrodes are used with complex voltages having direct current components. The shape of the figures gives the cyclic relations of current and voltage. Retraced linear segments show ohmic resistance between corresponding voltages. Horizontal segments on the voltage axis appearing with intermittent currents represent decay of voltage between electrodes when no current flows. Horizontal translation represents cumulative back electromotive force. Vertical translation measures change in direct current component resulting from ohmic change and back electromotive force. Changes in slope of elongated figures indicate changes in impedances which are predominantly resistance. These changes can be recorded cinematographically.

By the method outlined it is now possible to measure, during electrical stimulation, those properties of the living tissue stimulated which determine the passage of the electrical impulses, and hence to know what constitutes the actual electrical stimulus affecting the tissue.

Serum calcium in Eck fistula dogs. LUDWIG G. LEDERER (introduced by Lathan A. Crandall, Jr.). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Illinois.

The average of 34 determinations of fasting serum calcium in 8 Eck fistula dogs is 9.35 mgm. per 100 cc., as compared with 10.65 in 54 determinations in 18 normal dogs. In the Ecks 37 per cent of all fasting values lie below 9.1, as contrasted with 7.4 per cent below 9.1 in normal animals.

When calcium was administered orally as the diphosphate, both normals and Ecks show little significant change in serum calcium. Following oral administration of calcium lactate (5 normal, 8 Eck) or gluconate (13 normal, 6 Eck) in 10 gram doses the rise in serum calcium is definitely less in Eck fistula dogs than in normals. In gastrectomized dogs (courtesy Dr. A. C. Ivy) the rise following oral calcium lactate tended to be greater than that in normals, after gluconate it was approximately equivalent to the normal rise.

Intravenous calcium chloride was given to normal and Eck fistula dogs for one hour periods by Woodyatt pump. When 17.3 mgm. of calcium per kilo were injected the average rise in normals was 5.0 and in Ecks 4.4. When 8.6 mgm. per kilo were given the rise in normals was 2.4, in Ecks 1.6.

Injection of 7 units per kilo of parathormone in 5 normal dogs produced an average rise of 1.4 mgm., the same dose in 7 Ecks caused an increase of 0.96 mgm.

The evidence from serum calcium studies indicates that there may be a deficiency of calcium in animals with chronic liver damage, with more rapid utilization or deposition when its salts are given in excess, and more difficult mobilization by parathormone. The data on gastrectomized dogs indicate that the absorption of calcium in the fasting animal is not influenced by the gastric secretion.

✓ *The effect of bulbocapnine upon the fatigue of skeletal muscle in situ.* C. E. LEES and A. C. ROBERTS (by invitation). Department of Physiology, The George Washington University Medical School.

The pronounced tension of skeletal muscle after bulbocapnine intoxication fails to show a steady state in the nerve muscle preparation in situ.

Decerebrate cats were arranged so that the optimal stimulus frequency and strength could be delivered to the peripheral end of the popliteal nerve and the tension of the soleus and gastrocnemius muscles recorded. Stimuli could also be delivered to the muscle directly.

Comparisons of the fatigability of the nerve muscle preparation before and after bulbocapnine were made.

The results indicate that the fatigue produced after bulbocapnine is more truly a muscle fatigue than a myoneural junction fatigue. It is suggested that the factors responsible for these results are directly concerned with an altered vascularity of the muscle.

Strength duration curves of the over- and underexcitable nerve muscle apparatus and some consequences concerning clinical application of chronaximetry.

F. H. LEWY (introduced by H. C. Bazett). University of Pennsylvania.

Although it is generally accepted that the strength duration curves of the normal nerve muscle apparatus are approximately hyperbolae this has not yet been proven under pathological conditions, i.e., in over- and under-excitability.

1. By means of chronaximetrical examination of 825 lead workers subjected to different degrees of exposure it could be shown that the strength duration curves even of the over- and underexcitable nerve muscle apparatus equal approximately a hyperbola.

2. The hyperbolae of normal, over- and underexcitable individuals differ in the degree of their curvature. The legs of the hyperbola of an overexcitable muscle form a more rectangular curvature, those of an underexcitable a more obtuse curvature. Normal curves lie between the two extremes.

3. The most suitable symbol of characterizing an actual strength duration curve and indicating simultaneously its shape is the vertex of the hyperbola. The coördinate of the hyperbola—which may be called “vertex characteristic”—equals the square root of the product of rheobase and chronaxie or in other words of half of the quantity. By this method one single and easily comparable figure is obtained. This one figure characterizes unmistakably the actual strength duration curve including the rheobase.

4. Three simple formulae are given to calculate the “vertex characteristic” by help of three independant measurements, carried out at one and the same skin muscle point. The approximate coincidence of the three values seems to confirm that it was really the optimal point that was stimulated.

5. In cases of degeneration it may happen that not all points of the strength duration curve are situated upon one and the same hyperbola. The curve may be discontinuous, indicating that it consists of two distinct hyperbolae.

6. The inversion of Pflüger's law applies to the excitation time as well. Even before the galvanic threshold with the anode sinks below that with the cathode, the excitation time with the anode becomes shorter than that with the cathode.

The effect of cortin upon the experimental neurosis in sheep. H. S. LIDDELL, O. D. ANDERSON (by invitation) E. KOTYUKA (by invitation) and F. A. HARTMAN. Department of Physiology, Cornell University, Ithaca. Eight years ago Liddell and Bayne observed, during motor conditioning

experiments, an enduring derangement of behavior in the sheep which closely resembled the "experimental neurosis" described by Pavlov. This case and others subsequently observed were characterized by extreme excitement, uneöoperative behavior, and spontaneous, nervous twitching movements of the conditioned forelimb. In every case the "neurosis" resulted from a conditioning routine to which the sheep could not adequately adjust itself. Subcutaneous injections of cortin in the neurotic sheep greatly increased the vigor of the conditioned flexions of the forelimb and at the same time decreased the frequency of the spontaneous movements of this limb. The animals were quieted and became more coöperative both outside and inside the laboratory. Cortin also increased the conditioned reflex in normal sheep. The above results are in accord with those obtained in man. The repeated subcutaneous injection of 1:200,000 adrenalin solution had an effect upon the behavior of the sheep exactly opposite to that of cortin. Adrenalin decreased the vigor of the conditioned reflex in both normal and neurotic animals, and in the latter it aggravated the nervous condition. When cortin relatively free from epinephrine was administered the beneficial effects were greater and cumulative. They persisted for more than 24 days after the last injection. An approximately quantitative method of analyzing the muscular activity was employed.

Characteristics of single motor unit responses in human muscles during various degrees of contraction. D. B. LINDSLEY (introduced by A. Forbes). National Research Fellow, Department of Physiology, Harvard Medical School.

Action potentials have been obtained by means of concentric needle electrodes from single motor units in several flexor and extensor muscles of the limbs of normal human subjects during graded series of contractions and in fatigue. The action potentials have been recorded by means of a portable, six stage, transformer-coupled amplifier, a DuBois oscillograph and a moving film camera.

The response of a unit at any specific intensity of contraction is characterized by a definite amplitude, wave form and rhythm. The rhythm is remarkably regular during moderate or strong contractions but is less so during weak contractions. The characteristic wave form and amplitude of a unit's responses remain constant when the strength of contraction is varied. Rhythmic responses are the rule but when a contraction is very weak occasional or arrhythmic responses are sometimes observed.

As the intensity of contraction increases, the frequency increases, with gradual accession of other units. The frequency of simultaneously active units may vary widely, depending upon their relative tension, but usually all units active close to the electrodes show a similar frequency.

The lowest regular rates at which single units respond during the weakest voluntary contractions are about 4 per second. The highest rates with very strong contractions are about 40 per second. Evidence from patients with progressive muscular atrophy, with but few active units remaining, maximally weighted, suggests an upper limit of 50 per second. The majority of contractions (excluding very weak and very strong) involve unit frequencies from 10 to 25 per second.

Single units have been maintained in continuous activity at a moderate rate for 30 minutes without indication of rotational activity or change in

rhythm, amplitude or wave form. Fatigue caused by contractions at constant intensity for a prolonged period shows responses of irregular and progressively diminishing amplitude. At near maximum intensities of contraction and during fatigue the responses of various units become grouped. In both cases the increased synchronization of responses is correlated with tremors of the mechanical record of tension.

The antidotal effect of picrotoxin in coma produced by the intravenous injection of barbiturates. C. R. LINEGAR (introduced by Theodore Kopppanyi). Department of Pharmacology of Georgetown University School of Medicine, Washington, D. C.

The antidotal effect of picrotoxin has been amply demonstrated both in long and short acting barbiturate poisoning by Maloney, Tatum and others. This drug was used in this series of experiments to prolong the life of poisoned animals after fatal doses of barbiturates in order that the fate of these soporifics in high dosages could be studied. While the antidotal effect of picrotoxin was confirmed, it was possible to study this action under new conditions, e.g., after intravenous administration of massive doses of barbiturates and occasionally in barbital coma where the heart beat and respiration had stopped.

The maximum intravenous doses administered during an injection period of about twenty minutes were 2200 mgm. of sodium barbital per kilogram and 260 mgm. of nembutal per kilogram. The maximum antidotal dose of picrotoxin, which was many times the convulsive dosage, was 38 mgm. per kilogram in the dog. The massive amounts of barbiturates as well as of picrotoxin were given in divided doses. In each case picrotoxin was administered in sufficient amounts to keep the animal in a state of slight convulsions which was indicated by minor twitchings of the extremities, mouth and tongue.

It was found that picrotoxin prolonged the life of all poisoned animals and if the animals recovered it shortened the recovery time. In long acting barbiturates picrotoxin prolonged the life, restored the animal's reflexes, but had no life-saving effect unless the barbital depression was completely controlled by picrotoxin injections because of the difference in duration of action of the two drugs. When respiration and heart beat had stopped after the administration of 1 to 2 grams of sodium barbital per kilogram, picrotoxin and strychnine injections restored both, strychnine being effective even after picrotoxin had no more effect. In one dog the intravenous injection of 20 mgm. of strychnine per kilogram produced prompt recovery of respiration but no convulsions in a dog which had received 1650 mgm. of sodium barbital and 29 mgm. of picrotoxin per kilogram.

Further work on the analysis of this antagonism is being carried out.

The reflex influence of the lower portion of the large gut on the tonus and movements of the empty stomach in dogs. E. R. LOEW (by invitation) and T. L. PATTERSON. Department of Physiology, Wayne University, Detroit.

It has been demonstrated that the tonus and the contractions of the empty stomach (man and dog) are temporarily inhibited by stimulation of nerves in the mouth, in the esophagus, in the stomach, and in the upper portion of the small intestine. It would seem probable from these results

that stimuli arising from the large gut might produce similar effects of physiological significance on the gastric motility. To determine if the tonus and hunger contractions can be influenced reflexly by stimulation of the large gut studies were made by the balloon method on dogs provided with gastric fistulae and the contractions were recorded by the new kymographic ink recording method (Patterson).

A small amount of pressure in an intrarectal balloon has no effect, but pressure of such magnitude that the rectum is filled and distended, as occurs when stuffed with feces causes a decrease of gastric tonus and inhibition of the gastric hunger contractions, and if the pressure is adequate it may lead to the defecation reflex. Furthermore, prolonged gastric inhibition results whenever the pressure in the intrarectal balloon becomes sufficient to produce a decided feeling of discomfort and restlessness in the animal. The effects following distention of the rectum are probably of reflex nature referable to viscero-sensations which are initiated in the large gut. Small amounts of water introduced into the large gut via a tube which passes through a slightly inflated intrarectal balloon, which serves as an anal plug, does not affect the character of the gastric movements. These results should be of value in interpreting some of the conditions arising from constipational disorders.

Action of cobra venom on the nervous system. DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md.

Recent attempts to use cobra venom in safe dosage to relieve pain of inoperable carcinoma prompted the present inquiry into the mechanism of its analgesic action. Experiments on effect of cobra venom on various parts of the nervous system yielded the following findings: 1. Cobra venom is not a local anesthetic for sensory nerve endings, as shown by experiments made on frogskin and by the "wheat" method on guinea pigs. 2. Direct application of the venom to nerve fibers and nerve trunks of frogs and cats reveals no local anesthetic effect. 3. Considerable quantities of cobra venom added to Locke solution containing surviving smooth muscle preparations of bladder, intestine and other organs do not readily paralyze either the sympathetic or parasympathetic nerve endings, as indicated by their active response to subsequent treatment with epinephrine, pilocarpine or atropine. 4. Suitable doses of cobra venom, injected intraperitoneally in mice, tend to antagonize the cerebral convulsions produced by camphor. 5. On stimulation with faradic current, the pain threshold is raised in guinea pigs injected intraperitoneally with cobra venom. 6. Experimental psychological studies on rats in a circular maze prove that injections of small doses of venom produce a primary stimulation while larger doses are followed by depression of cerebral and neuromuscular responses. 7. A series of clinical cases of inoperable carcinoma treated with injections of suitable doses of cobra venom were definitely relieved of pain, and in patients requiring morphine the dosage of the narcotic could be greatly reduced or discontinued altogether. All the evidence in hand indicates that cobra venom relieves pain in much the same way morphine does, through its action on the cerebrum, but without exerting the narcotic effects of the latter.

Action of saligenin and mono-brom saligenin on the central nervous system.

DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

The two outstanding physiological properties of mono-brom saligenin, or Bromsalizol, are its antispasmodic action on smooth muscle and local anesthetic effect on sensory nerve endings. Solutions of Bromsalizol, 0.5 per cent, are also mildly antiseptic. Since saligenin and Bromsalizol are derivatives of salicylic acid and the salicylates are known to exert antipyretic and analgesic effects, experiments were made to ascertain whether saligenin and Bromsalizol also possess such properties. Bromsalizol exerts a mildly sedative, but not narcotic, action on the cerebrum, much like that produced by the salicylates, as indicated by the following findings: 1. Injections of from 5 to 10 mgm. mildly depressed the behavior of rats trained in a circular maze. 2. Bromsalizol, administered by mouth to rats, guinea pigs and rabbits with hyperpyrexia, produced a moderate lowering of the temperature. 3. Injected intraperitoneally in mice, rats and guinea pigs, mono-brom saligenin antagonized the convulsant action of camphor injections. This action was not produced by saligenin itself, indicating that the bromine component of Bromsalizol was concerned in the anticonvulsant action. 4. In a series of controlled clinical tests with Bromsalizol on epileptic patients, this compound, substituted for phenobarbital, produced definite sedation. 5. In a series of arthritic patients, Bromsalizol relieved pain and was better tolerated by the stomach than ordinary acetyl salicylic acid. Of the isomeric mono-brom saligenins, the mono-brom ortho saligenin, or Bromsalizol, was the most effective.

Mechanism of psyllium action on intestines. DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Two factors are responsible for the laxative action of psyllium seeds. The first and more important is the mechanical stimulation of intestinal walls produced by swelling of the mucilaginous constituents of the seeds in water, a property which is not destroyed by heat. The author's experiments also reveal that even bread made from dough in which the seeds had been incorporated, in spite of the oven temperature to which they were exposed, produced more copious stools when fed to rats than similar bread without psyllium seeds. Experiments with chloroform, petrol ether and alcoholic extracts of psyllium seeds revealed that there was a second factor regarding their purgative action to be considered. Hydro-alcoholic extracts contain oils and other extractives which definitely stimulate contractions of isolated surviving intestinal loops in warm oxygenated Locke solution *in vitro*. These oils or extractives probably act as adjuvants to, or synergistically with, the mucilaginous ingredients in promoting bowel movements.

Action of some antiseptics on muscle oxydase. DAVID I. MACHT and HILAH F. BRYAN (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

The influence of twelve antiseptics was studied on muscle oxydase according to Thunberg's method of determining the time for decolorization of a standard solution of methylthionine in specially constructed vacuum tubes. Freshly prepared muscle juice from albino rats killed by arteriot-

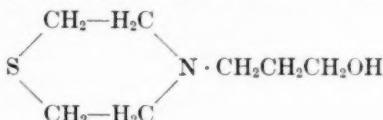
omy, or bleeding, was employed in all the tests. The inhibition of enzyme activity by antiseptics did not run parallel to their antiseptic power but was an excellent index of their toxicity for tissues and organs. Thus, phenol, diphenyl and strong alcohol inhibit the activity of muscle oxydase out of all proportion to their bactericidal power. The most interesting finding was the marked difference between organic and inorganic mercurial antiseptics in relation to their inhibition of oxydase action and of bactericidal potency, respectively. The organic mercurials, dibrom-oxymercuri-fluorescein, or Mercurochrome, and monohydroxy-mercuri-di-iodo resorcin-sulphonphthalein, or Merodiceein, inhibit oxydase activity very little even in concentrations greater than those needed to kill bacteria, whereas bichloride and biniodide of mercury markedly depress enzymatic activity even in very dilute solutions. Mercurochrome, in solutions of 1:250, does not appreciably inhibit the activity of muscle oxydase; and even concentrations of 1:100 are but slightly depressant; whereas bichloride, in solutions of 1:5000, greatly weakens the activity of oxydase; and the oxide cyanide of mercury kills it altogether after only short exposure. These findings are consistent with the low toxicity of dibrom-oxymercuri-fluorescein for living tissues and organs, on the one hand, and with the great toxicity of bichloride for the same test objects, on the other. The Thunberg method proved useful in detecting adulterations of Mercurochrome with small quantities of inorganic mercury compounds. As little as one part of inorganic mercury to twenty thousand of legitimate solution could be detected in this way.

Activity of muscle oxydase in relation to method of slaughter. DAVID I. MACHT and HILAH F. BRYAN (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Muscle oxydase was studied by the Thunberg method of decolorization of a standard methylene blue solution in special vacuum tubes. Fresh muscle juice from albino rats was used. It was found that the method employed in killing the animal influenced the activity of the muscle oxydase. The animals were killed by arteriotomy, by injuries to the brain either by percussion with a blunt instrument or decerebration by a sharp probe driven through the skull, by electrocution and by asphyxiation with ether, chloroform, hydrogen, ethylene, pure nitrous oxide and monoxide gas. Comparison of the muscle oxydase after arteriotomy, or bleeding, with that prepared from animals killed by other methods revealed that injuries to the brain markedly influenced the enzyme, inhibiting its activity in some cases and accelerating it in others. Electrocution inhibited the activity of oxydase. Ether asphyxia resulted in a slight depression, while chloroform and ethylene gas markedly depressed enzymatic action. Hydrogen gas produced no change; nitrous oxide accelerated it; and of all the gases studied carbon monoxide inhibited muscle oxydase to the greatest extent.

Physiological action of thiomorpholine propanol derivatives. DAVID I. MACHT and LAWTON A. BURROWS (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., and Laboratory for Organic Chemistry, Johns Hopkins University, Baltimore, Md. (Read by title.)

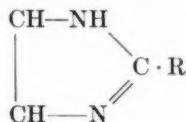
Thiomorpholine proponal, having the following formula, was synthesized;



sized; and three derivatives of this compound were prepared by introducing acetyl, benzoyl and para-amino-benzoyl groups in place of the hydroxyl. In view of the mild local anesthetic property of morpholines (Gardner and Haenni: J. Am. Chem. Soc. **53**: 2763, 1931) and thiomorpholines (Macht: Proc. Soc. Exp. Biol. and Med. **31**: 234, 1933), these compounds were examined for such action by the frogskin and guinea pig "wheal" methods. The only one found to produce local anesthesia was the benzoate derivative. The para-amino benzoate exhibited very little local anesthetic action. The hydrochloride of thiomorpholine proponal itself was also ineffective. Injected intravenously in cats under ether anesthesia, all the compounds produced transient depression of circulation and respiration.

Physiological action of some glyoxalidine compounds. DAVID I. MACHT and HENRY C. CHITWOOD (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., and Laboratory for Organic Chemistry, Johns Hopkins University, Baltimore, Md. (Read by title.)

Five compounds were synthesized and studied physiologically. The general structure of these is expressed by the formula, R being replaced by



the methyl, ethyl, propyl, normal butyl and normal amyl radicles, respectively. Of these, the only one known before was the methyl compound called *lysidin*, which has been studied as a solvent for uric acid (Ladenburg, Ber. xxvii, 3, 2952) and must not be confused with the amino acid named lysin. Aqueous solutions of these compounds were tested on seedlings of *Lupinus albus*, goldfish, tadpoles, mice, rabbits and cats. Contrary to the usual pharmacological experience, the toxicity of the five compounds, in general, decreased with the increase in length of the alkyl radicle. Goldfish and tadpoles survived in solutions of 1:5000 but succumbed in the order named when placed in solutions of 1:1000. Ten milligrams of the methyl compound, administered intraperitoneally, killed mice, but the higher members of the series were not so toxic. Phytotoxic indices also showed that the latter were less poisonous for plants. Half a gram per kilo of any one of these compounds, given by stomach, was not toxic for rabbits and did not impair their kidney function. The methyl compound, however, increased the acidity of the urine. Ten milligrams, injected intravenously in cats under ether, produced a transient fall in blood pressure but little effect on the respiration. In all the reactions studied, the lower compounds were more toxic than the higher.

Toxicity of reptilian blood. DAVID I. MACHT and MARY ELEANOR DAVIS (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

The senior author has described in numerous previous publications his phytopharmacological studies on normal and pathological *human* blood. In connection with this research, comparative physiological studies were made on blood of all kinds of animals. Blood of twelve kinds of mammals, of five kinds of birds and six species of fish, and of frogs, tested on *Lupinus albus* seedlings, gave approximately the same phytotoxic index as human blood; namely, from 70 to 75 per cent. The blood of reptiles proved a notable exception. Three species of turtle, *Chrysemis*, *Pseudomys* and *Terrapina*, exhibited a toxic reaction, giving an index much lower than 70 per cent. The same was true of blood specimens from the alligator, gila monster and chuckawalla, all of which were very toxic for living seedlings. Of special interest were the results obtained with snakes' blood. Blood sera from two species of rattlesnake, from copperheads and from the "non-poisonous" garter snake, black snake and king snake were tested on plants and small animals. They were all highly toxic, as indicated by inhibition of root growth and by toxic reactions of mice, injected with venom solutions, and by depressed behavior of similarly injected white rats in the circular maze. The results obtained indicate that "non-poisonous" snakes, although devoid of poison glands and fangs, possess in their blood a toxic substance not much less potent than that contained in the blood of rattlesnakes and copperheads.

The hypophysectomized-pancreatectomized monkey. WILLIAM MAHONEY (introduced by J. F. Fulton). Laboratory of Physiology, Yale University School of Medicine, New Haven.

The interdependence of pancreas and pituitary body has been emphasized by work on toads and dogs. Repetition of these studies on sub-human primates has established the existence of this endocrine interrelation in these forms. Following hypophysectomy in monkey, baboon and chimpanzee there occurred a pronounced fall in the blood sugar. This "hypoglycemia hypophysopriva" came on spontaneously if the carbohydrate supply was inadequate, and led to death in some instances. In others the onset was prevented by giving carbohydrates. The maintenance of a high environmental temperature (*ca.* 88°F.) helped remarkably to maintain the blood sugar level. Hypophysectomized monkeys are especially sensitive to cold, but the surprisingly low level of body temperature which they sometimes show (90-91°F.) can be accounted for only in part by the associated fall of blood sugar. The sugar tolerance curve of the hypophysectomized-pancreatectomized monkey starts low, and climbs very slowly without reaching even the minimum initial level of a diabetic curve.

A completely pancreatectomized monkey can live without administration of insulin if the entire pituitary body has been removed. Death supervenes, however, if the hypophysectomy is incomplete. Lesions of the hypothalamus (anterior, posterior, or tuberal) and application of a silver clip to the pituitary stalk fail to cause any demonstrable alteration of the blood sugar, although a transient glycosuria is sometimes encountered. None of these procedures replaces complete hypophysectomy in maintaining carbohydrate equilibrium after pancreatectomy.

In both dog and monkey after hypophysectomy and pancreatectomy a marked emaciation follows similar to Simmond's disease in man. Glycosuria is not noted unless carbohydrate intake is greatly in excess of the usual diet. In dogs any amount of protein can be ingested without subsequent glycosuria, but this has not been determined in the monkey, for the normal primate diet is low in protein. One hypophysectomized-pancreatectomized monkey has been living for over ten months without insulin.

Observations on blood pressure responses to electrical stimulation of the central end of the vagus. W. H. MARSHALL (introduced by A. B. Luckhardt). Department of Physiology, University of Chicago, and Department of Physiology, George Washington University.

Blood pressure responses to central vagal stimulation in cat and dog preparations were observed with a vacuum tube stimulator, permitting the use of rectangular stimulating pulses, the duration, intensity and frequency of which could be varied over a wide range. In the dog, a maximal sustained pressor effect of about 100 per cent elevation of arterial pressure could regularly be obtained with strong stimuli of low frequency, two to ten per second. The depressor effect was best obtained with weak stimuli of a higher frequency, but was transitory.

In the cat, the pressor effect was smaller or absent, the period of stimulation being succeeded by a typical depressor curve. Weaker stimuli of higher frequency produced a prompt and more sustained depressor response.

The responses obtained gave some additional suggestions of reciprocal action of the centers as regards pressor action.

Evidence that the vagus nerve is connected with the pressure responsive pressor end organs in the dog was obtained in one experiment.

A sensitive device for measuring insensible water loss. W. H. MARSHALL (by invitation) and E. C. ALBRITTON. Department of Physiology, The School of Medicine, The George Washington University. (Demonstration.)

The water vapor is estimated by measuring the relative humidity of the air drawn over the surface under observation. The relative humidity can be estimated with great accuracy over a wide range of sensitivity by means of wet and dry thermocouples, the flow of the air being regulated and measured by flow-meters of appropriate sensitivity. Variations of as little as 0.02 mgm. per liter per minute can be detected.

A study of the relative rate of absorption from normal and burned tissue. EDWARD C. MASON, H. A. SHOEMAKER and PEARL PAXTON (introduced by D. E. Jackson). Department of Physiology, University of Oklahoma School of Medicine.

Tannic acid was introduced as a treatment for burns on the theory that it precipitated the injured tissue and thereby prevented the absorption of toxic material. More recently, it has been claimed that absorption did not occur from burned areas and such claims were supported by observations following the injection of strychnine and phenolsulphonephthalein into burned areas and observing the rate of absorption.

This study is concerned with the rate of absorption following subcu-

taneous injections of 2.29 per cent solution of potassium iodide in normal and burned animals. We have observed the rate of absorption for 12, 24, and 36 hour periods and our results have led us to believe that absorption from burned areas does occur; also, that the rate of absorption of potassium iodide is as rapid, and in some cases more rapid, in burned than in normal animals.

The function of sulfur in various physiological processes in the flagellate, Chilomonas paramecium. S. O. MAST and D. M. PACE (by invitation). The Zoological Laboratory of the Johns Hopkins University. (Read by title.)

Chilomonas contains much starch, fat and protein. It can synthesize these and protoplasm from inorganic compounds, obtaining carbon from CO_2 and nitrogen from NH_4Cl . Eight elements are necessary. This synthesis is not dependent upon chlorophyl or light. The energy required is probably obtained from oxidation of ammonia.

As the concentration of sulfur increases from zero, in sterile solutions, the rate of fission increases from zero to a maximum of $4 \pm$ divisions per day, then decreases. This obtains for sulfur in Na_2S , $\text{Na}_2\text{S}_2\text{O}_3$, Na_2SO_3 , Na_2SO_4 , cysteine and glutathione reduced ($-\text{SH}$) or oxidized ($\text{S}-\text{S}$) but the optimum concentration of sulfur varies greatly. There is no consistent correlation between the extent of oxidation of the sulfur and the rate of fission.

If sulfur is absent, growth and fission continue, starch is synthesized and fat is formed.

If sulfur is in optimum concentration and acetate is absent, starch and fat decrease and growth ceases, but the chilomonads are active and fission continues. They die in $6 \pm$ days, very small and practically free from starch.

If sulfur and acetate are absent, starch decreases and fat increases but growth ceases and the rate of fission and the activity of the chilomonads are much reduced. They die in $4 \pm$ days, large, with much fat and little starch.

In solutions containing insufficient sulfur, decrease in rate of fission ending in death is due to retardation in oxidation, resulting in inadequate release of energy and excessive accumulation of fat (fatty degeneration). In solutions containing insufficient carbon in usable form (CO_2 , acetate, sugar, etc.) it is due to retardation in synthesis of starch, resulting in starvation. In solutions containing excess sulfur or carbon in usable form it is due to some unknown inhibitory action of these substances on the processes involved in cell division.

Carbon in usable form is necessary in the environment for synthesis of starch. Fat is formed from starch. Neither carbon nor sulfur is necessary in the environment for cell division. Sulfur functions as a catalyst in oxidation of fat and thus inhibits accumulation of fat; and it is used in synthesis of cysteine present in all protoplasm.

Hemoglobin studies in chickens. GEO. H. MAUGHAN. Department of Physiology and Biochemistry, Cornell Medical College, Ithaca.

Studies made in our laboratory, as well as work done by others, show that the blood of chickens has a lower hemoglobin content than that of man (the figures range between fifty and eighty per cent). It is also

true that variations, between wide limits, occur in what seem to be normal birds; and averages on day old chicks from different flocks of the same breed may be ten per cent or more apart.

Fluctuations in hemoglobin content of individual hens are marked, often changing ten per cent in a week. One of the most constant differences appears in the amount of hemoglobin of the blood of laying hens, as compared to younger chickens, cocks and hens out of production. Results clearly indicate, as has also been shown by Cook and Harmon, that hens in production have a lower hemoglobin content than those out of production. The differences may be as much as thirty per cent. Our averages were approximately ten per cent lower for hens laying three or four eggs a week. Another interesting fact seems to have been demonstrated; namely, as the egg yolks are forming previous to the onset of production the blood hemoglobin often falls. This may also account for the hemoglobin fluctuations in producing hens where cycles of high followed by cycles of low egg production occur.

Rachitic chickens have a low hemoglobin content. The amount is forty-three to eighty-five per cent of that found in normal chickens. In a number of tests on very rachitic birds the hemoglobin was as low as five grams per 100 cc. of blood (14.5 grams per 100 cc. blood as a standard for man).

Additions of 0.3 per cent iron sulfate to the regular diet did not seem to affect the hemoglobin of a small group of laying hens kept in individual confinement. The addition of iodine (10 mgm. KI a day) to the normal diet (Cornell laying mash) did not raise the hemoglobin in a similar group of twelve hens.

Extinction: local stimulatory inactivation within the motor cortex. WARREN S. McCULLOCH (by invitation) and J. G. DUSSE DE BARENNE. Laboratory of Neurophysiology, Yale University, New Haven.

Repetition of electrical stimulation of any focus of the motor cortex (*macacus*) with an interval of a few seconds gives appearance or increase of response, i.e., facilitation; with a longer interval (15 seconds or more) absence or diminution of response, i.e., extinction.

Electrical pulses of various forms and frequencies, large and small, polarizable and non-polarizable electrodes, monopolar and bipolar stimulation, all produce extinction. It appears with deep and light narcosis, under dial or ether and has even been found by a special device for stimulation in animals recovered from narcosis.

The conditions underlying extinction are complicated, for the characteristics of both first and second stimulation of any pair, and the interval between them, are significant. With equal stimulations greater intervals, durations, frequencies and pulse energies favor extinction; smaller—facilitation. Cathode ray oscillograms have shown that the actual stimuli were electrically identical, although extinction was manifest. With unequal stimulations an antecedent giving facilitation with one subsequent, may give extinction with another and—conversely—a subsequent giving facilitation with one antecedent may give extinction with another.

Subliminal as well as supraliminal stimuli are effective in producing extinction. This and the facts given above preclude any simple explanation of extinction as fatigue or exhaustion.

Extinction is to be distinguished from so-called cortical inhibition,

which is the obliteration of an existing muscular response through stimulation of a cortical focus.

Extinction is a sharply localized phenomenon, for circumthermocoagulation and circumcainization of the motor focus leave extinction present, whereas minute displacement of the electrode between two stimulations suffices to replace it by facilitation. After destruction of the outer three layers of the cortex by laminar thermocoagulation extinction persists, whereas it is superseded by facilitation when the remaining layers are coagulated or when the whole thickness of the cortex is removed and the electrodes are applied to the corona radiata.

Extinction, therefore, is a cortical phenomenon, the extinction of the response on electrical stimulation of the cortex being due to localized inactivation of those cells in the inner two layers directly beneath the electrode. All available evidence implicates the large and giant pyramidal cells.

An amplifier for minute direct currents. A. R. MCINTYRE, Department of Physiology and Pharmacology, University of Nebraska Medical College, Omaha. (Demonstration.)

This instrument consists of an internally shielded box containing an amplification circuit employing the well known F.P. 54 vacuum tube. Terminals are provided for the galvanometer and battery connections. The input lead is air-insulated and contains no switch. The arrangement combines great convenience of operation with high sensitivity and currents of the order of 10^{-12} amperes may be quickly and conveniently measured. The only additional equipment consists of a sensitive galvanometer and a 12 volt storage battery. The instrument may be used in the determination of H ion concentration and is especially useful when a glass electrode of the Morton type is used.

The effect of reduced atmospheric pressure upon the leucocyte count. OVID O. MEYER (introduced by M. H. Seevers), University of Wisconsin Medical School.

Normal and splenectomized rats kept in a chamber at a barometric pressure ranging from 422 to 282 mm. Hg for periods of one to twenty-eight days developed early leukocytosis followed by persistent leukopenia with a concomitant increase in hemoglobin, erythrocytes and reticulocytes. No consistent changes in the differential formulae were noted, relative neutropenia did not occur in most experiments. The results in the two groups of animals were similar.

The results in rats were in some degree confirmed in guinea pigs.

Previous reports in the literature of the effect of reduced oxygen tension upon the leucocyte count have lacked uniformity. It is thought that these results may help to clarify the problem since the factor of acclimatization may explain why leukocytosis has been noted by some observers, leukopenia by others.

The influence of progressively increasing, toxemic liver damage upon the dextrose tolerance curve. I. ARTHUR MIRSKY (by invitation) and SAMUEL SOSKIN, The Metabolic Laboratory, Department of Physiology, Michael Reese Hospital and the Department of Physiology, University of Chicago.

We have previously shown that one of the chief factors in determining the course of normal dextrose tolerance curves is a homeostatic reaction of the liver, in which this organ decreases its supply of sugar to the blood in response to the influx of exogenous sugar. It has also been shown that the abnormal dextrose tolerance curves which appear in toxemic states are due to an interference with this homeostatic liver mechanism.

The present report deals with the successive dextrose tolerance tests obtained from the time of the intravenous injection of diphtheria toxin until the resulting death. The results indicate that the tolerance curves so obtained are influenced by a progressive liver damage, and that the sequence of events may be divided into three stages: First, a "hyper-irritability" of the liver which limits the degree of inhibition produced by the test sugar and produces "diabetic" tolerance curves. Second, a decrease in the "irritability" back towards normal so that normal tolerance curves are again obtained, but at a higher blood sugar level. Third, a "hypo-irritability" of the liver which rapidly progresses to complete loss of function with hypoglycemia. During this stage, as in the hepatectomized animal, either "supernormal" or "diabetic" curves may be obtained depending upon the amount of sugar used for the test.

It is concluded that this cycle of events has an important bearing upon the interpretation of the dextrose tolerance curve, and upon the correlation between tests of hepatic function and estimated liver damage.

A pseudafferent reflex evoked by injections into the left coronary artery and the peripheral paths of the pain-fibers which are concerned. ROBERT M. MOORE and ALBERT O. SINGLETON, JR. (by invitation). Department of Surgery, University of Texas, Medical Branch. (Read by title.)

In decerebrate cats, after etherization had been discontinued, the thorax was entered under artificial respiration, the pericardium opened and a thread passed beneath the stem of the left coronary artery proximal to its bifurcation.

Manipulations of tissues adjoining the artery, e.g., pinching, gave rise to reflex movements of the extremities and of the head and jaws. Tension on the thread so as to occlude the arterial trunk, if prolonged for 10 or 15 seconds, resulted in similar reflexes. No reflex effects occurred when only one branch of the artery was occluded.

Injection of irritating solutions distal to the thread evoked immediate and marked reflex activity, exaggerated respiratory movements constituting a prominent feature. Isotonic KCl served to elicit this effect, suggesting that the pain-endings affected are as sensitive as those previously described in relation to the branchings of other arteries.

Intra-arterial injection of 0.75 cc. 20 per cent lactic acid caused instantaneous and very marked reflex activity associated with a rapidly progressing, black discoloration of practically the entire wall of the left ventricle and of a large part of the wall of the left auricle. This was followed in 5 or 10 seconds by acute dilatation of both chambers. The reflex activity was immediate and marked in every one of 15 animals in which the left thoracic sympathetic trunk was intact, although the animals had been subjected to unilateral or bilateral cervical sympathectomy or vagotomy. In contrast were the results in 9 animals in which the left thoracic sympathetic trunk had been resected from above the stellate ganglion to the 7th

rib. Although the cervical trunks and the right thoracic trunk were intact, in none of these animals did reflex effects occur until ventricular dilatation had become extreme and there was advanced asphyxia.

It is apparent, then, that all pain-fibers ending in the region of the left coronary artery pass to the spinal cord by way of the rami of the upper thoracic ganglia of the left side. As yet no injections have been made into the less accessible right coronary artery.

Neuro-muscular phenomena in response to variation in calcium and potassium concentrations in the cerebro-spinal fluid. F. J. MULLIN (by invitation), W. M. LEES (by invitation) and A. B. HASTINGS. Physiological Laboratory and the Lasker Foundation for Medical Research of the Department of Medicine, the University of Chicago, Chicago.

The effect of changing the concentration of calcium and potassium ions in the cerebro-spinal fluid on the state of activity of peripheral muscles has been studied by irrigation of the cisterna magna. Fluids were injected into and removed from the cisterna magna through a small rubber tube, one millimeter in diameter, put in place by the technique described by Jacobs. Muscle tension was measured by a cathode ray oscillograph or by a string galvanometer. The lead-off electrodes were placed in the neck muscles and the reaction of the muscles recorded photographically.

Control observations were made in which cerebro-spinal fluid was withdrawn and then reinjected. No change in peripheral muscle tension was observed in these control experiments. When a balanced salt solution containing inorganic ions in the concentrations in which they exist in cerebro-spinal fluid was substituted for the cerebro-spinal fluid of the dog, there was no change in the state of activity of the musculature studied. When solutions of identical composition, except for the fact that they contained no calcium, were injected a marked increase in muscular activity resulted. This effect was readily abolished by restoring the calcium ion concentration of the cerebro-spinal fluid to normal (1.0 mM per liter). When 0.5 cc. of sodium citrate (77 mM per liter) was injected directly into the cisterna magna a marked generalized muscular response was observed, due to the fact that the calcium ion concentration of the cerebro-spinal fluid was greatly reduced. Solutions containing 12 mM of potassium per liter brought about changes in muscle activity similar to but not so marked as those containing calcium. This effect was abolished by solutions containing a normal concentration of potassium ions.

Effects of certain analgesic drugs on cutaneous, tactile and pain sensitivity. F. J. MULLIN (by invitation) and ARNO B. LUCKHARDT. Physiological Laboratory of the University of Chicago. (Read by title.)

Determinations of cutaneous, tactile and pain sensitivity were made by the use of Von Frey hairs every half hour, for $2\frac{1}{2}$ to $3\frac{1}{2}$ hours, following the administration of supposedly analgesic drugs. Eleven marked spots on the face and hand were tested in irregular order, and the values obtained were checked and compared with control determinations on the same spots before the drug was taken.

Morphine sulphate ($\frac{1}{4}$ - $\frac{1}{2}$ grain), alcohol (300-375 cc. of 20 per cent), trichlorethylene, and, to some extent, codeine sulphate (1- $1\frac{1}{2}$ grains), all reduce the sensitivity to pain without affecting appreciably tactile sensitivity. In several instances the sensitivity to pain experienced on

recovery from the drug was greater than normal. Morphine caused difficulty in micturition.

Aspirin (2-3 grams), luminal (5-10 grains), sodium bromide (4-8 grams), acetanilid (0.3 gram), and calcium gluconate (10 grams), were without appreciable effect on pain and tactile sensitivity. Various alleged potentiators of aspirin, such as calcium gluconate and magnesium chloride, did not change the action of the aspirin.

The influence of the cardiac sympathetics and adrenin on the phenomenon of ventricular escape. L. H. NAHUM and E. C. HOFF. Laboratory of Physiology, Yale University, New Haven.

When adrenin is injected intravenously nodal and ventricular ectopic rhythms may be seen during the resulting cardio-depressor discharge. If only one vagus is intact, auricular ectopic rhythms appear as well. In such experiments the depression of upper structures by the vagus allows lower cardiac areas, whose rhythmicity is enhanced by the adrenin, to "escape" with the formation of ectopic rhythms. It seemed desirable therefore to study the part played by adrenin and sympathin normally present in the body in the phenomenon of ventricular escape since the combined, but unequal, activity of the vagus and sympathetics on the heart may cause many of the ectopic beats found so often in normal and abnormal hearts.

Monkeys (*Macaca mulatta*) were anesthetized lightly with sodium amytal, the vagi exposed in the neck, and electrocardiograms taken during stimulation of each vagus by an inductorium with the secondary coil at 10.5 cm. The stellate ganglia and both adrenal glands were then removed and thereafter at varying intervals electrocardiographic records were obtained during stimulation of the vagi with the same current strength. After adrenalectomy and bilateral stellate ganglionectomy "ventricular escape," although not totally abolished, was profoundly diminished. It is concluded that adrenin and the sympathetic nerves to the heart play an essential rôle in ventricular escape. Probably many if not all ectopic rhythms involving escape phenomena are promoted by adrenin and the cardiac sympathetics.

The chemical analysis and enzyme content of intestinal juice. E. S. NASSET and H. B. PIERCE. Department of Vital Economics, University of Rochester, Rochester, N. Y.

Intestinal juice was collected from transplanted sections of the upper jejunum at hourly intervals. It was analyzed for solids, ash, chloride and carbonate, and chloride in the ash was also determined. The activity of sucrase and peptidase was estimated by following quantitatively the digestion of sucrose and peptone. Samples were collected at hourly intervals for 7 hours on fasting and food days, and the effect of food on chemical composition and enzyme content was noted.

There were marked variations in the content of solids and enzymes from hour to hour, particularly following food, whereas the ash content was fairly constant and the chloride content remarkably constant in individual animals.

The reaction of the rat's stomach to acetylcholine. H. NECHELES and R. FRANK (by invitation). Michael Reese Hospital, Chicago.

In view of the reported appearance of acetylcholine in the stomach following vagus stimulation, it was of interest to determine the vasomotor reaction of the stomach to this drug. Rats' stomachs were perfused through the celiac axis with Ringer's solution to which varying amounts of acetylcholine were added. As controls rabbits' ears and hind legs of rats were perfused in the same way.

Results. Rat's stomach: Stronger solutions of acetylcholine (1:50,000) produced either a small reduction in venous outflow or a small increase; concentrations of 1:100,000 were followed by a large decrease in venous outflow (-45 to -90 per cent). Concentrations of 1:1,000,000 always produced a slight decrease of venous outflow (-17 to -26 per cent). There was no visible contraction of the stomach when it was perfused with acetylcholine-Ringer. Rabbits' ears and hindlegs of rats never showed a diminution of venous outflow after acetylcholine; some showed even a marked increase (+24 to +50 per cent). Adrenalin diminished venous outflow from stomach, hind legs and ears, while sodium nitrite always raised it.

The non-filament and filament neutrophil count during emotional excitement.

L. B. NICE and H. L. KATZ (by invitation), D. FISHMAN (by invitation) and D. L. FRIEDMAN (by invitation). Department of Physiology, Ohio State University.

The cytology of the blood of rabbits was studied in the quiet state and during emotional excitement (fear, anger, pain).

It was found that a definite leucopenia took place during the latter condition in these rabbits. The leucopenia proved to be a neutropenia. This led us to determine the relative numbers of non-filament (young neutrophils) to filament (old cells) in the blood of these rabbits by a modification of the method of Cooke and Ponder.

In a study of thirty cases in the normal state the average number of the non-filament cells was 22 per cent, while during excitement it was 23 per cent. These numbers are closely similar indicating that the same proportion of non-filament and filament neutrophils disappeared from the peripheral blood (ear vein) of our rabbits during excitement.

There was no indication of an increased supply of new neutrophils being set free from the red bone marrow during excitement.

Effects of local cooling of the floor of the fourth ventricle on respiration. HAYDEN C. NICHOLSON and DAVID BREZIN (by invitation). Department of Physiology, University of Michigan, Ann Arbor.

Moderate local cooling of the posterior portion of the floor of the fourth ventricle resulted in a decrease in the duration of the expiratory phase of respiration with little effect upon inspiration, thus increasing respiratory rate. More marked cooling caused a definite increase in the duration of the inspiratory phase, resulting in a decrease in respiratory rate usually below the original value. These effects were obtained with either intact or sectioned vagi.

These effects of local cooling were then used in an attempt to analyze the actions of various modifiers of respiration. Carbon dioxide (12 to 14 per cent) caused a much greater increase in respiratory rate during cooling than under normal conditions. This increase was most marked when cooling resulted in a well marked decrease in the expiratory pause with

only a slight prolongation of inspiration. Although with the vagi blocked, and the temperature of the medulla normal, carbon dioxide usually causes a slowing of respiration, during cooling it caused as marked an acceleration with the vagi blocked as when they were intact. Low oxygen seemed to be equally effective in stimulating respiration with normal and sub-normal temperatures. The slowing of respiration produced by inflation of the lungs was less marked if the inflation was performed during the administration of carbon dioxide and still less marked during cooling. Provided this slowing was not completely abolished by cooling, it was lessened still more by cooling and carbon dioxide combined. The same was true of the acceleration of respiration produced by deflation of the lungs except that the effect of cooling was somewhat less marked than in the case of inflation. Carbon dioxide was found to cause marked acceleration of respiration during inflation of the lungs, less marked acceleration at neutral lung volume and least marked acceleration or even slowing during deflation, agreeing with the previous work of Gesell and Moyer. The same effects of carbon dioxide were observed during cooling unless the cooling abolished the effects of inflation and deflation upon respiratory rate, in which case the effect of carbon dioxide upon respiratory rate was the same at all lung volumes.

The importance of electrical shunts in contact with the heart in determining the electrical field. FELIX OCKO (by invitation), ISAAC GUTMAN (by invitation), EDWARD SIGMAN (by invitation) and LOUIS N. KATZ. Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, Chicago.

We have recently shown that introducing electrical insulators between the heart and certain adjacent structures greatly alters the standard three lead electrocardiogram. In the present study it was found that: 1, a metal conductor surrounding the heart and in contact with it decreases the amplitude of the standard three lead electrocardiogram; 2, a shunt placed on a small region of the heart alters the standard three lead electrocardiogram; 3, a shunt connecting a region of the heart to the chest wall alters the standard three lead electrocardiogram; 4, a punctate lead directly from the heart when joined to a lead at a distance from the heart gives a practically monophasic curve; the monophasic curve changes to a diphasic curve when electrical shunts are placed in contact with the punctate lead on the heart.

These results indicate that the amount of electrical shunting of heart currents is of considerable importance in determining the electrical field of the body.

Gastric motility of the terrestrial mollusc, Ariolimax californicus, as influenced by temperature, insulin and glucose. T. L. PATTERSON. Department of Physiology, Wayne University, Detroit.

For experimental purposes the stomach of this animal was divided into two parts, the cardiae-intermediate and pyloric portions, the latter of which exhibits the more vigorous contractions when placed in oxygenated Ringer's solution after one end has been attached to a fixed support and to the other a light heart lever, for recording the contractions. Preliminary experiments on the intact animal by the balloon and x-ray methods indicate that the rate of the peristaltic waves over the pylorus are practically

identical with those of the isolated stomach and that the movements are continuous. The pyloric portions of stomachs removed from animals in a state of repletion and in the fasting condition were tested by this method, and it was found, that the contractions obtained from the stomach of a fasting animal were more regular and in prolonged fasting about five times stronger than those obtained from the stomach of a well nourished animal during digestion. Temperature coefficients showing the relation between contractions and temperature are maintained either when the stomach passes from a lower to a higher temperature or vice versa, which is in support of van't Hoff's rule.

The limited amount of work done with insulin on invertebrate animals makes it desirable to determine its influence along with glucose on the fasted and well nourished stomachs. The introduction of 0.067 cc. of U-10 "Iletin" (insulin Lilly) with a hypodermic needle directly into the pyloric stomach obtained from a well nourished animal produced a marked augmentation of the gastric tonus, whereas the same amount of a 25 per cent solution of glucose produced an inhibitory effect on the fasted stomach which was undergoing gastric activity. These results of insulin and glucose on the stomach of the invertebrate animal are in agreement with those obtained on dogs and would seem to indicate the importance of carbohydrates as a controlling factor of the gastric hunger mechanism.

New improvements on the ink recording system for kymographic registration.

T. L. PATTERSON, R. E. SCANTLEBURY (by invitation) and J. A. GIJSBERS (by invitation). Department of Physiology, Wayne University, Detroit. (Demonstration.)

In our laboratory we have devised a piece of apparatus which has several attractive features that are of interest both to the teacher and the student, as well as combining economy with success. The new ink recording pen is simple in construction, the results are gratifying, and the expense is almost negligible.

The pen is made from the quill of a large stiff feather obtained from the wing or tail of an adult chicken. The end that is attached to the body of the fowl is tapered off to a point and at the same time the firm pith in the end of the quill is preserved, which acts as a plug to keep the ink from flying out of the ink pocket. A flexible shaft electric or dental drill is used for drilling a small opening in the quill, which is made just above the firm pith near the tapered end, and the pen point is split from this opening through the center of the point and trimmed under a binocular microscope. An opening is then cut on the upper side of the quill, just in front of where the solid, white pith begins that extends through the feather and the loose scales of pith below are removed by means of a small brass or copper wire with the end bent to form a hook. This forms an ink pocket into which a special blue, kymograf ink is introduced with a curved pipette. In filling, all air must be expelled from the ink pocket. About an inch of the end of the quill containing the solid, white pith is preserved for holding. The pen may be used on all types of recording instruments (manometers, tambours, signal magnets, tuning forks, etc.). It is also most important that a heavy grade of kymograph paper be used. For photographic reproduction a red ink may be substituted. All steps in pen making will be demonstrated.

Methemoglobin versus sulphemoglobin following ingestion of acetanilid.

W. D. PAUL and B. B. CLARK (introduced by F. M. Smith). Laboratory of Pathological Chemistry and the Department of Internal Medicine, University Hospital, The State University of Iowa, Iowa City.

The older literature and current textbooks state that methemoglobinemia results from the ingestion of acetanilid. Harrop and Waterfield (1930) reported 10 cases from which they concluded that only sulphemoglobin and not methemoglobin was formed. Vogel (1924) reported one case of sulphemoglobinemia resulting from exposure to para-amido-phenol.

We have examined the blood of many patients who gave a history of taking acetanilid or Bromo-Seltzer in fairly large quantities for a long period of time. No methemoglobin was found. The four cases reported here which have been more carefully studied gave such histories. No methemoglobin was demonstrated (Clark and Gibson bichromate method, 1933). The blood of 3 of 4 cases showed a definite absorption band in the red between C and D which was unaffected by ammonium sulphide. The oxygen unsaturation of the blood was determined in three cases by taking the difference between total hemoglobin calculated from the iron content and active hemoglobin by oxygen capacity. The per cent of unsaturation was 28.6, 18.4, and 9.6. We feel sure that the abnormal pigment is sulphemoglobin.

One of the cases had taken acetanilid for 15 years. On admission to the hospital acetanilid was withdrawn for about 10 days; the oxygen unsaturation at the end of this time was 1.5 per cent. She was then given acetanilid beginning with 6 grains daily and increasing to 12 grains daily. After 20 days the oxygen unsaturation was 9.6 per cent; no methemoglobin was found; only a faint absorption band between C and D could be seen.

We have observed several cases of unquestionable methemoglobinemia following exposure to nitrobenzenes. One patient fully studied had 60 per cent methemoglobin after exposure to dinitrobenzene and promptly recovered after administration of glucose. We feel that methemoglobin is definitely formed following exposure to nitrobenzene derivatives only and not to acetanilid or similar chemicals.

The influence of digitalis-like glucosides on energy liberation and efficiency in the isolated mammalian heart. HOWARD C. PETERS (by invitation) and MAURICE B. VISSCHER. Department of Physiology, College of Medicine, University of Illinois, Chicago.

Controversies over the question of the action of digitalis glucosides on the dynamics of heart muscle must be settled, it seems, on the basis of energy studies. One previous report (Ruhl and Wiegler, Arch. Exp. Path. and Pharmacol. **175**: 665, 1934) has recorded studies on total energy liberation in relation to the effect of strophanthin. The experimental procedure did not permit decisive conclusions because diastolic ventricular volume was not controlled. Starling and Visscher (J. Physiol. **62**: 243, 1927), showed that in any study of energy liberation by heart muscle the diastolic volume must be rigorously controlled.

Experiments have been conducted in which energy liberation at constant diastolic fiber length has been measured by means of oxygen consumption determinations. The method is essentially as described by Peters, Rea and Visscher (Proc. Soc. Exp. Biol. Med. **32**: 268, 1934).

After suitable control periods 0.000125 gram doses of scillaren B, or

similar small doses of strophanthin, were introduced into the circulating blood of hypodynamic preparations. The diastolic volume was maintained constant by adjustment of the venous return while oxygen consumption and arterial pressure were continuously recorded. Volume output and rate were determined frequently.

Scillaren B promptly increased both the oxygen consumption and the efficiency of the heart at constant diastolic fiber length. The efficiency may be doubled following such treatment. The larger increases in efficiency occurred in cases where the heart had failed badly before the scillaren injection.

The other digitalis glucosides act much more slowly and a definite effect has not been observed within as short a period as with scillaren B.

The increased energy liberation found after scillaren at constant diastolic ventricular volume indicates that these substances induce changes similar to those following calcium salt injection (Peters, Rea and Visscher, loc. cit.), or following adrenalin. These results in general show that for the diastolic ventricular volume to determine energy liberation the chemical conditions must be kept constant. The initial fiber length still determines the energy liberated in contraction, but on a new scale, in the presence of adequate concentrations of these substances.

Determinations of plasma volume in dogs during experimental fever and during exposure to heat. JAMES O. PINKSTON (by invitation) and MAGNUS I. GREGERSEN. Laboratories of Physiology, Harvard Medical School.

In view of the importance attributed to circulatory adjustments (Bazett, 1931), and to alterations of blood concentration (Barbour and colleagues) in the regulation of body temperature, it seemed desirable to investigate the plasma volume changes.

Determinations of plasma volume (blue dye T-1824, serum technique and spectrophotometric analysis) in 4 dogs before and at the height of experimental fever (typhoid-paratyphoid vaccine) showed in two animals no change; in two others, an increase of 5 to 10 per cent which was reflected in the dilution of the plasma proteins (determined by refractometer). The febrile response was characterized in each case by shivering and by an increase of body temperature (0.34 to 1.22°C.).

During exposure to heat (39 to 41°C. for 1 hour) three dogs which panted rapidly showed a measurable decrease of plasma volume and an increase of plasma protein. After exposure there was a prompt return to normal. In none of the 8 experiments, including one in which panting was prevented by a bandage over the mouth, was there an increase of plasma volume. Identical results were obtained on sympathectomized dogs. In two normal human subjects under similar experimental conditions there was a slight decrease of plasma volume.

Is the Weber interpretation of auditory mechanics correct? A. G. POHLMAN.

Department of Anatomy, Creighton University, Omaha, Nebraska.

E. H. Weber in 1851 proposed a solution for the mechanics underlying an immersion receptor for air sounds. His explanation was so simple and so obvious that it was adopted practically without question and is the fundamental basis for all theories on sound analysis. The interpretation may be separated into five interrelated postulates and the evidence will

be submitted that four of these postulates are incorrect. The deletion of the Weber explanation makes it unnecessary to discuss in detail why none of the theories on sound analysis fit the experimental evidence.

Experiments on auditory mechanics. A. G. POHLMAN. Department of Anatomy, Creighton University, Omaha, Nebraska. (Demonstration.)
1. The reflection back of air sounds by water.
2. The conduction of vibration in solids to water.
3. The correlation between air and bone conducted speech.
4. A new improvement on the acoustic fan.
5. A method of listening to the reversed action of the sound conduction apparatus.

New and improved apparatus for teaching and research. W. T. PORTER and F. H. PRATT. Dover, Massachusetts, and Boston University School of Medicine. (Demonstration.)

Devices, integral with a long-paper and a single-cylinder electric kymograph, for immediate shift from the vertical to the horizontal position, with provision for the support and accurate adjustment of a new form of smoking-lamp; an electrically driven piston pump for artificial respiration; swivel and fine-adjusting double clamps for recording apparatus, and a stainless-steel seeker adapted in strength for blunt dissection of dense fascia.

The persistence of acacia in the blood after intravenous injection of acacia solution. M. H. POWER, NORMAN M. KEITH and E. G. WAKEFIELD (by invitation). Section on Clinical Metabolism and Division of Medicine, Mayo Clinic, Rochester, Minnesota.

We have determined acacia by precipitating it with acetone from trichloracetic acids filtrates of serum, and subsequently oxidizing the precipitate quantitatively with potassium dichromate. The procedure in our hands constitutes a simple, convenient, and accurate method for the determination of acacia. Estimation of acacia in the serum of 15 patients at various times after injection has revealed that in some instances the acacia has remained for remarkably long periods of time. In one case almost three years had elapsed since the last injection, yet acacia was still present in small amount, 25 mgm. per 100 cc. of serum. Such instances usually represent subjects who have received large or multiple injections. As renal insufficiency was not present to marked degree in any of the subjects studied, it seems probable that these observations do not signify retention by the kidney, but rather the slow liberation of acacia from deposits temporarily formed in the body. Recent work of Andersch and Gibson on the accumulation of acacia in the liver and spleen supports this view.

A possible route whereby such a deposit could be gradually eliminated from the liver cells into the blood stream, aside from simple diffusion back into the blood capillaries, lies through the lymph drainage to the thoracic duct. In experiments in dogs in which 0.6 gram of acacia per kgm. of body weight was injected 24 hours after recovery from ether anesthesia, we have found that the thoracic duct lymph contains appreciable quantities of acacia, 300 to 400 mgm. per cent within 3 hours.

A preparation for the study of single synaptic junctions. C. LADD PROSSER (introduced by Hudson Hoagland). Clark University and Harvard Medical School.

Each sensory hair on the caudal lobes of the crayfish contains one sensory ending. When a hair is flexed, one impulse is observed by electrical recording in the afferent nerve after a latency of 3 to 4σ . This sensory mechanism shows a refractory period of less than 0.01 second. When the hairs are stimulated repetitively the response synchronizes for a long time with the stimulus up to approximately 50 per second, and falls to a constant level after 5 to 10 stimuli at 50 to 100 per second.

When impulses are recorded anterior to the caudal ganglion no response is obtained when less than 3 or 4 adjacent hairs are stimulated. There is a spatial summation ratio of approximately 4 afferent impulses to 1 efferent. Four impulses from hairs separated by several cut ones are not adequate. The response anterior to the ganglion shows a latency of 7 to 10σ , hence 4 to 6σ are consumed in passing through the synapse in the ganglion. These synapses are refractory for approximately 0.1 second, with considerable variation for different fiber-synapse complexes. The efferent response synchronizes with afferent impulses only at frequencies up to 8 or 10 per second. No alteration in size or latency in the efferent impulses has been noted after recovery from a previous discharge.

In contralateral nerves of the caudal segment efferent responses are also obtained. These show a ratio of approximately 2 afferent impulses to 1 efferent and synchronize with afferent volleys up to 25 or 40 per second.

The ganglionic (synaptic) delays for the tracts followed by proprioceptive and tactile responses through abdominal ganglia of the crayfish are 4 to 6σ . The conduction velocities for these fibers vary from 3 to 8 m. per second.

The effect of parathyroid hormone upon the serum calcium and calcium excretion of adrenalectomized rats. L. I. PUGSLEY (introduced by J. B. Collip). Department of Biochemistry and Pathological Chemistry, McGill University.

Adrenalectomized rats receiving one per cent sodium chloride in their drinking water were found to respond with a greater calcemia and calciuria than normal rats under the same conditions and receiving the same amount of parathyroid hormone per day.

A study of mineral metabolism in arthritis under treatment with vitamin D. C. I. REED, M. L. HATHAWAY (by invitation), and H. C. STRUCK (by invitation). Department of Physiology, College of Medicine, University of Illinois, Chicago. (Lantern.)

Balance experiments on seven arthritic patients showed marked retention of calcium and phosphorus. Treatment with daily doses of vitamin D in the form of viosterol 10,000X, ranging from 150,000 to 1,000,000 U. S. P. units, tended to restore a balance which was coincident with clinical improvement.

After-potentials and recovery curve of C fibers. C. H. RICHARDS (by invitation) and H. S. GASSER. Department of Physiology, Cornell University Medical College, New York City.

As compared with A fibers, the positive after-potential on C fibers (bullfrog splanchnic) is greatly exaggerated (confirming Bishop). Corresponding to the larger after-potential there is a modification in the recovery curve. Two aspects of recovery may be tested separately: ability to respond and threshold of excitation. The absolutely refractory period lasts 0.010 second at 24°C. The excitability then increases rapidly and becomes approximately normal after 0.03 second. After this time, instead of continuing to normal or becoming supernormal, the excitability decreases again and restoration to the initial state does not take place until the positive after-potential has subsided. In its configuration the excitability curve greatly resembles the curves for A fibers poisoned by yohimbine, as obtained by Dr. H. T. Graham.

In fresh C fibers the negative after-potential is only visible as a variation on the positive after-potential without there being any actual negativity. Veratrinization of the nerve causes the negative after-potential to grow with a well defined rising phase so that the latter is well set off from the spike. In some nerves veratrinization produces an after-potential higher than the spike. (The spike is recorded after conduction and, because of temporal dispersion, does not give a true index of the axon spike height.) As negativity develops there is a change in the recovery curve. After the first sharp rise in excitability there is no subsequent decline but the excitability goes on increasing so that a well-defined supernormal phase is developed. This lasts as long as the negative after-potential (about 20 seconds) and is followed by a subnormal phase accompanied by a positive potential lasting at least a minute.

Ability to respond is tested with supermaximal shocks. The absolute height of the crest attains normal when the spike intervals (not stimulus intervals) are separated by 0.1 second. Inasmuch as the spikes rise from the level of the still continuing positive after-potential, they are higher than normal by an amount equal to the magnitude of the latter. The sizes of the responses are thus supernormal at a time at which the excitability, as tested by thresholds, is subnormal.

Effectiveness and specificity of prolactin in the induction of the maternal instinct in virgin rats. OSCAR RIDDLE, ERNEST L. LAHR (by invitation) and ROBERT W. BATES (by invitation). Station for Experimental Evolution, Carnegie Institution.

Virgin albinos isolated from males at 30 days and tested at 75 days old usually begin to retrieve and care for young at 5 to 12 days after the first of a series of daily subcutaneous injections of prolactin. In some cases the maternal instinct is exhibited in definite form within 24 or 48 hours after first injection. In virgins with open vaginae previous "priming" with prolan or follicle-stimulating hormone is unnecessary, as is also a period of "concaveation" with newborn young; in virgins with closed vaginae the "priming" process is probably necessary, though neither estrin nor progestin induces the maternal instinct. Neither prolan, growth hormone, mare serum nor follicle-stimulating hormone, the latter containing some thyrotropic (and luteinizing?) principle, induces the maternal instinct in these rats. Whole anterior pituitary extract, containing prolactin, is effective in most cases; in some virgin rats neither the unfractionated extract nor prolactin has induced retrieving and care of young during a 20 to 40 day period of injection.

Prolactin induced activities which express maternal behavior in virgin rats.

OSCAR RIDDLE, ERNEST L. LAHR (by invitation) and ROBERT W. BATES (by invitation). Station for Experimental Evolution, Carnegie Institution. (Motion pictures, demonstration.)

Motion picture records show the retrieving of young by prolactin injected virgin rats. Because of disturbing effects of flood lights—necessary to making the pictures—the care of the young in nest is less well recorded. The absence of retrieving activities in uninjected virgins, and in virgins injected with some hormones other than prolactin, is shown. The very young pigeon squab and mouse is retrieved as is the very young rat.

Inhibition of flexor reactions on homolateral cortical stimulation. D. McK. RIOCH and A. ROSENBLUETH. Departments of Anatomy and Physiology, Harvard Medical School.

Electrical stimulation of the cerebral cortex around the cruciate sulcus in cats anesthetized with dial was found to produce inhibition of flexor responses in both the homolateral legs. This phenomenon was studied in *M. tibialis anticus*. The hind leg under examination was partially denervated, the tibia fixed by drills, and the muscle attached to a spring myograph recording on a kymograph. Contractions were evoked either by stimulation of the contralateral motor cortex or by producing a series of flexor reflex twitches with single shocks applied to the saphenous nerve. Stimulation of the homolateral cortex during the contractions resulted in a partial relaxation or a decreased contraction. The inhibition was greater or less, depending on the relative strengths of the stimuli used. On cessation of the homolateral stimulation the contractions tended to return to their original force. When the inhibitory stimulus preceded the excitatory the responses to the latter were markedly delayed and smaller than the control observations.

The threshold for the homolateral inhibitory response was lower over the premotor area (area 6 of Brodmann) than over the motor (area 4). No inhibitory results were obtained from other regions of the cortex. The effect persisted after section of the corpus callosum and after bilateral section of the dorsal spinal roots from L3 to S2 inclusive. It was not secondary to other movements of the homo- or contralateral limbs, as it was frequently obtained in the absence of movements attributable to the homolateral stimulation (e.g., homolateral extension, contralateral flexion).

A similar, but less pronounced, inhibition was obtained by stimulation of the cerebellum.

The effect of ouabain upon the type of electrocardiogram resulting from specific muscle lesions. JANE SANDS ROBB, M. S. DOOLEY (by invitation), J. G. F. Hiss (by invitation) and R. C. ROBB (by invitation). Laboratories of Pharmacology and Medicine, Syracuse University College of Medicine, Syracuse, New York. (Read by title.)

The prior injection of ouabain does not prevent the appearance of those characteristic modifications of the electrocardiogram due to lesions in individual muscles, nor does subsequent therapeutic dosage alter these results. Toxic doses, however, accentuate the existing depression of the S-T interval due to ischemia of the deep sino-spiral muscle, but minimize the R-T elevation produced by injury to either of the two superficial muscles or to the deep bulbo-spiral.

The pathway of the excitatory process in the mammalian ventricle. JANE SANDS ROBB and ROBERT C. ROBB (by invitation). Syracuse University College of Medicine, Syracuse, New York.

Simultaneous direct and indirect leads from mammalian hearts have been recorded with a three-galvanometer arrangement (devised by the Cambridge Instrument Company). Thus the events at any two points on the heart may be accurately related to a standard lead II, without introducing error of time or potential.

Times of initial negativity are found to increase in an orderly manner, from apex to base, on condition that the electrodes be placed upon a given muscle in a series *parallel* to the fibres of that muscle. If a transverse cut be made between any two contacts in the above series, the arrival of an intrinsic wave at the more cephalad point will be delayed. Furthermore, if the entire muscle cross-section be involved, the intrinsic wave fails to arrive at that point further from the apex. Conduction rates have been calculated from point to point oriented along muscle strands. In both dogs and monkeys, the rate approximates 2400 ± 100 mm./sec.

These data support the hypothesis that, on the surface of the heart, the excitatory process is conducted by some pathway parallel to fibre direction, from apex toward the base, in the muscles studied.

Experimental cardiac muscle lesions in the monkey. JANE SANDS ROBB and ROBERT C. ROBB (by invitation). Syracuse University College of Medicine, Syracuse, New York. (Read by title.)

The same electrocardiographic changes known to occur in dogs, consequent to coronary ligations affecting specified muscles, have been obtained in the Macacus rhesus monkey. The upward or downward displacement of the R-T (or S-T) segment is in each case qualitatively according to expectation.

The duration of the S-T interval (but not of the Q.R.S.) is always increased as the result of such muscle injuries. This appears to differ significantly from the normal relation: $S-T = 0.216 \sqrt{\text{cycle length}}$ (Bazett and Sands, Annals Clin. Med. 5: no. 2, 1926).

The distribution of the coronary vessels to the ventricular muscle bands.¹ JANE SANDS ROBB and ROBERT C. ROBB (by invitation). Syracuse University College of Medicine, Syracuse, New York. (Demonstration.)

Dissections of human and dog hearts have been prepared to demonstrate four of the ventricular muscle bands and the blood supply to each. It is seen that certain of the coronary branches are distributed predominantly to one muscle. The internal portions of the superficial sino- and bulbo-spiral muscles are mainly supplied by branches of the ramus anterior descendens. The left head of the deep sino-spiral is supplied by the R. collateralis descendens anterior primus and its right portion by the R. ventriculi dextri anterior, R. marginis acuti and the R. ventriculi dextri posterior from the right coronary. The deep bulbo-spiral is supplied by small unnamed branches of the left circumflex interspaced between those larger branches which have been already described and named by Spalteholz.

¹ Grateful acknowledgment is made to the Hendricks Fund of Syracuse University, to the Committee on Scientific Research of the American Medical Association and to the Ella Sachs Plotz Fund for support of these experiments.

Chemical and physical characteristics of synovial fluid mucin. MARIAN ROPES (introduced by Walter Bauer). Massachusetts General Hospital. The few reports in the literature characterizing normal synovial fluid mucin vary markedly.

In this investigation mucin obtained from the joints of normal cattle has shown consistently the following characteristics. It is a grayish or yellowish amorphous substance. It is soluble in serum, weak alkali and mineral acids, insoluble in alcohol and ether, and practically insoluble in water. The viscous solutions on precipitation give a ropey, elastic mass. In solutions of varying pH, there is marked swelling below pH 3.0 and above pH 4.5, but very little swelling at pH 3.8-4.2. Precipitation with phosphoric acid is incomplete above pH 4.2. Molisch, biuret and xanthroproteic tests are positive. The mucin contains no phosphorus. Sulphur is present in a concentration of about 1.0 per cent. Reducing substances of approximately 8 per cent are present after hydrolysis. The mucin from different lots of fluid is of constant composition and, after purification, is unaffected by repeated reprecipitations.

4th reprecipitation.....	C 49.71	H 6.60	N 12.45
10th reprecipitation.....	C 49.57	H 6.55	N 12.13

The concentration of mucin in cattle fluid as determined by a single precipitation with acetic acid varies between 20 and 30 mgm. of mucin nitrogen per 100 cc. These values include other proteins precipitated with the mucin. Separation of these substances by repeated reprecipitation with acid gives values of approximately 15 mgm. of mucin nitrogen.

Thus, it would appear that a mucin of constant composition has been isolated from normal bovine synovial fluid.

Analyses of fluid obtained post mortem from patients with no joint disease give higher mucin values than those of cattle fluid. The mucin nitrogen in these fluids as determined by a single precipitation varies between 40 and 100 mgm.

The mucin concentration in pathological joint fluids is even higher. A single precipitation gives mucin nitrogen values between 50 and 260 mgm. per 100 cc. Reprecipitation with acetic acid, however, gives much lower values—30 to 60 mgm. of mucin nitrogen per 100 cc. The large amount of protein carried down with the first mucin precipitate is apparently due to the extremely high total protein content of such fluids.

Reflex responses of the nictitating membrane. A. ROSENBLUETH and H. G. SCHWARTZ (by invitation). Departments of Physiology and Anatomy, Harvard Medical School.

In adrenalectomized cats under urethane anesthesia the nictitating membrane shows a moderate tonic contraction, demonstrable by the relaxation which follows section of the cervical sympathetic. Central stimulation of somatic and autonomic nerves evokes reflex contraction or relaxation. Afferent nerves known to have reflex effects on the blood pressure, the heart rate and the respiration have been found likewise effective in eliciting responses from the membrane. These nerves are muscular, cutaneous and visceral afferents—the latter including both sympathetic and parasympathetic nerves.

Afferent stimulation of the vagi usually induces inhibition, more marked if applied during a reflex contraction elicited from another afferent, e.g., the sciatic. The fibers in the vagus responsible for this inhibition are probably the depressor afferents, for section of the vagi and denervation of the

carotid sinuses augment strikingly the reflex contraction from other nerves, e.g., the sciatic.

The other afferents studied, cutaneous, muscular and visceral, contain both excitatory and inhibitory fibers, for an excitatory rebound at the end of stimulation is obtained with adequate stimuli. The reflex responses are bilateral and of approximately equal magnitude. A summation of the effects from two afferents occurs when they are stimulated simultaneously. Facilitation and occlusion in the responses may thus be demonstrated.

The after-discharge of the centers concerned may be of long duration—several minutes—as in other autonomic reflexes.

Simultaneous records of the nictitating membrane and the blood pressure show that, although the responses are usually parallel in sign, relative magnitude and duration, it is possible to abolish this parallelism by suitable stimuli, thus revealing the independence of the two reflexes and showing that the characteristics of the membrane reflexes, e.g., the prolonged after-discharge, are not an indirect effect of blood-pressure changes.

The reflexes, indicative of generalized activity of the sympathetic nervous system, constitute a relatively simple area in which several features and properties of reflexes in general, and of autonomic reflexes specifically, may be studied.

The resynthesis of phosphocreatine after muscular contraction. JACOB

SACKS and WILMA C. SACKS (introduced by C. W. Edmunds). Department of Pharmacology, University of Michigan.

The changes in phosphocreatine and lactic acid content that occur in the first part of the recovery period after muscular activity consisting of a tetanus of 30 seconds' duration have been studied in the gastrocnemii of cats and rabbits, under conditions of intact blood and nerve supply. It was found that the removal of lactic acid is rapid at first and that the rate of removal soon decreases. During the time in which lactic acid is being removed, phosphocreatine is resynthesized from its hydrolysis products. A definite relation was found between the rates at which these two processes take place. One milligram per cent of phosphocreatine-P is resynthesized from its hydrolysis products for each 2.5 mgm. per cent of lactic acid removed. This is equivalent to the removal of equivalent amounts of acid and base at pH 5.6. The findings thus support Fiske's theory that the function of phosphocreatine hydrolysis in contracting muscle is that of buffering lactic acid.

In the first minute of recovery in the rabbit gastrocnemius, 10 mgm. per cent of phosphocreatine-P is formed; in the second minute, 6 mgm. per cent. It has previously been found that during a 5 second tetanus, this muscle forms 60 mgm. per cent of lactic acid. From the values for the heat of formation of lactic acid from glycogen (180 cals. per gram), and for the heat of hydrolysis of phosphocreatine (approximately 400 cals. per gram of P), determined in Meyerhof's laboratory, calculations have been made of the rate at which phosphocreatine resynthesis must take place during contraction, on the basis of Lundsgaard's hypothesis. From these figures it is calculated that, in order to satisfy this hypothesis, phosphocreatine-P must be resynthesized *during contraction* at the rate of about 325 mgm. per cent per minute. This is 30 to 50 times the rate actually found during the recovery period. These data are considered to cast doubt on the validity of the Lundsgaard hypothesis.

Carbohydrate changes during recovery from muscular contraction. JACOB SACKS and WILMA C. SACKS (introduced by C. W. Edmunds). Department of Pharmacology, University of Michigan. (Read by title.)

The gastrocnemius muscle of rabbits was tetanized for 30 seconds with blood supply and innervation intact, and then allowed to recover. The changes in glycogen, lactic acid and hexosephosphate content were followed throughout the recovery period until the lactic acid content had returned practically to the resting level.

It was found that the hexosephosphate which had formed during the tetanus was reconverted to glycogen and phosphocreatine at the rate of about 1 mgm. per cent of P per minute. The rate was somewhat less than this toward the end of the recovery period.

More than half the lactic acid accumulation was removed during the first five minutes of recovery; after this the removal became progressively slower.

Carbohydrate balances showed no evidence that any of the lactic acid which disappeared from the muscle during the recovery period was converted to glycogen within the muscle. The total carbohydrate content of the muscle became progressively less throughout the recovery period, and the loss was parallel to the loss of lactic acid. This finding makes it highly improbable that the "Meyerhof cycle" plays any part in the metabolism of muscular work in the mammal. The only means available to the muscle for disposing of lactic acid not oxidized is diffusion into the blood stream.

In the early part of the recovery period, a loss of carbohydrate, over and above the loss of lactic acid, becomes evident. This extra loss of carbohydrate can be accounted for, partly by lactic acid formation after the end of contraction, and partly by the extra oxygen consumption during the recovery period.

The enzymic synthesis from thyroid peptone of an artificial protein which relieves myxedema. WILLIAM T. SALTER. Collis P. Huntington Memorial Hospital, Boston, Mass. (Read by title.)

Thyroglobulin, prepared from human thyroids, was subjected to peptic digestion and, after removal of (combined) thyroxin, a solution of diiodotyrosine peptone was obtained free from protein. This could be filtered under pressure through a standard "Cellophane" membrane. After concentrating the peptone, it was subjected to a peptic synthesis which reversed the original digestion process. The mass of the product was, in one instance, over four hundred times the mass of the crystalline pepsin used.

The resulting product resembled natural thyroglobulin. It failed to dialyze through the standard "Cellophane" membrane. Its minimal solubility was sharply defined about pH 5.5. It was precipitated by trichloracetic acid in a concentration of 2 per cent. It was coagulated by heat; and the coagulum, although very insoluble in dilute hydrochloric acid alone, was readily digested and dissolved on the addition of pepsin at pH 1.5.

The nitrogen content was about 13 per cent (as against 15 per cent in the peptone used). The iodine content varied between 0.2 per cent and 0.3 per cent. As judged by modern analytical methods (Blau; Harrington and Randall), an appreciable amount of this colloidally-bound iodine was ap-

parently in the form of thyroxin. Five separate preparations of the artificial protein have been made. In terms of iodine, the maximal yield was 30 per cent of the original peptone iodine.

The artificial protein was found on clinical test to relieve human myxedema as effectively as thyroglobulin in equivalent-iodine dosage. This fact, together with the chemical resemblance of the artificial protein to natural thyroglobulin suggested that protease synthesis of thyroid hormone within the gland may be the physiologic process by which iodine is stored as colloid. An analogous artificial protein was prepared from iodinated horse serum, by digestion, followed by synthesis. It is interesting that this last product failed to relieve myxedema.

Excess oxygen usage reaction of silicotic individuals in exercise. BENJ. H. SCHLOMOVITZ, A. B. THOMPSON (by invitation) and L. GRANT GLICKMAN (by invitation). Clinical Laboratory, Veterans Administration Hospital, Milwaukee, Wisconsin.

Individuals with generalized pulmonary silicotic fibrosis were put through standardized moderate exercise for one minute in atmospheric air. Two 10 pound dumb-bells were raised from the floor to the shoulders and back to the floor, twenty-five times. Following the exercise the oxygen consumption was determined for 6 successive minutes, with a type of closed circuit oxygen metabolism apparatus that is widely used for basal determinations in clinical work. When the pulmonary fibrosis was dense (as in second or third stage chronic silicosis), then the excess oxygen usage differed from the normal in being low in the three minutes following exercise, and in having nearly similar values in the first and second minutes. This is true in what are apparently uncomplicated cases. The respective influence of collateral pulmonary and cardio-vascular pathology, thyroid dysfunction, and tachycardia, is being investigated. The data indicate that this method can be used as a lung function test per se.

The oxygen cost of a clinical function test. BENJ. H. SCHLOMOVITZ and A. B. THOMPSON (by invitation). Clinical Laboratory, Veterans Administration Hospital, Milwaukee, Wis. (Read by title.)

The basal oxygen consumption of healthy individuals was determined; then before, during, and after a standardized moderate exercise the subject breathed directly into the Benedict-Roth recording clinical metabolism apparatus. The one-minute exercise involved 25 floor-to-shoulder movements of two 10 pound dumb-bells, or roughly 5000 foot pounds. Determinations were made continuously for 6 minutes following the exercise. Determinations to date disclose that for the total seven minutes the excess oxygen consumption per 1000 foot pounds and per square meter of the subject's surface area, ranges from 256 cc. to 323 cc. The significance of rapid heart rates, age, weight, previous activity, etc., is being studied.

The intrinsic regulation of the circulation in the parietal cortex of the cat. CARL F. SCHMIDT. Laboratory of Pharmacology, University of Pennsylvania.

Using a thermoelectric method previously employed to investigate circulatory phenomena in the medulla and hypothalamus (This Journal 108: 241, 1934; 110: 137) a similar investigation is being made in the parietal regions of cats narcotized with pentobarbital or chloralose, cura-

rized, and subjected to constant artificial respiration. Results so far obtained show definite vasoconstrictor innervation via both cervical sympathetics; responses to faradic stimulation of these nerves are more constant and sometimes more marked than they were in the hypothalamic region. Surface (pial) vessels evidently respond much more actively than vessels 2 to 3 mm. within the cortex; the former show the sharp, promptly reversible constriction previously noted in extracranial tissues (muscle, tongue, etc.), while in the latter the constriction is usually slow in development and only partly reversible or irreversible thus resembling the vessels of the hypothalamic region. Stimulation of the vagodepressor and carotid sinus nerves has so far shown no sign of a direct influence on the parietal circulation. Attempts at exciting an intrinsic vasomotor innervation by direct faradization of the central nervous axis or by asphyxia have had a completely negative outcome here, as in the hypothalamic area. Adrenalin has shown no sign of a direct constrictor action on parietal vessels. Responses to chemical influences are of the same general nature as those observed in the hypothalamus or medulla: CO_2 -excess and oxygen-lack cause vasodilatation, the former being usually more effective than the latter; decrease in CO_2 causes constriction; increase in oxygen likewise causes constriction, much more consistently and powerfully than was the case in hypothalamus or medulla. Acid (NH_4Cl , HCl) is weakly vasodilator, but no sign of vasoconstriction from bicarbonate has so far been disclosed. Surface and deeper vessels behave almost identically in their responses to these chemical influences, and section of the cervical sympathetic and vagodepressor nerves does not modify the effects. So far it appears that the blood-vessels of the parietal cortex of the cat are more subject to vasoconstrictor nerve control than those of the hypothalamic region (or medulla), but much less so than the pial vessels of the parietal region; that oxygen has a much more marked constrictor effect upon parietal blood-vessels than upon those of the hypothalamus or medulla; in other respects the results resemble closely those obtained in the other areas. Further experiments are being made.

Properties of nerve proteins. FRANCIS O. SCHMITT and RICHARD S. BEAR
(by invitation). Department of Zoology, Washington University,
St. Louis.

Since the oriented structures in nerve indicated by thermal shortening, by swelling and by x-ray diffraction studies appear to be of protein nature, a study was made of the chemical and optical properties of nerve proteins. To avoid the necessity of lengthy dehydration and lipid extraction and to work as nearly as possible on pure axis cylinder material the claw and leg nerves of lobsters were used. The nerves were cut into bits and repeatedly extracted at pH 6.5 to 7.5. This procedure yielded a rather unstable nucleoprotein. Further extraction at pH 9-12 invariably gave another nucleoprotein with properties essentially similar to the first. After complete removal of these fractions a different nucleoprotein could be extracted in solutions of pH 13 to 14. The nerve dissolves practically completely at pH 14. These nucleoproteins appear to be essentially similar to those described for mammalian central nervous system by Levene, Shkarin and McGregor whose results have been confirmed by us with cow spinal cord and pig embryo brain.

To determine whether these proteins comprise the longitudinally oriented structures in nerve, the various nucleoproteins were spun into fibers by forcing their solutions through fine capillaries into coagulating media. Fibers were spun successfully from each of the nucleoprotein fractions of lobster nerve and of cow spinal cord. The dried fibers were quite strongly positively birefringent, a property typical of oriented protein fibers. The ease with which these proteins may be spun into birefringent fibers is evidence of the presence of long asymmetric micelles. However, only a portion of the fibers could have been oriented since a preliminary x-ray diffraction analysis (performed through the courtesy of Prof. G. L. Clark) showed only rings at approximately 23, 9.1-11.5 and 4.8A. The 9.1-11.5A spacing is also present in dried lobster and medullated nerve as found by Schmitt, Clark, Bear and Mrgudich (paper in press). Since the fibers were spun into alcohol, lipoids have been reduced to a minimum and further since the 9.1-11.5A spacing has not been found in lipoid materials, these experiments are evidence that the x-ray patterns in nerve are due at least partially to oriented nucleoprotein micelles.

The effects of posture upon cardiac output. JOHN C. SCOTT (introduced by H. C. Bazett). Physiology Department, Hahnemann Medical College, Philadelphia, Pa.

There has been considerable disagreement in the literature concerning the effects of posture on cardiac output. Grollman reported the output to be practically the same in the recumbent, sitting and standing positions and attributed some of the variations of previous workers to technical errors. Subsequent workers however, have continued to report variations with posture which disagree with Grollman's observations.

Most of the investigations of this problem were made with only one or two experiments on an individual for each of the three positions. It seems likely that incidental variations due to technical or physiological causes could mask the true changes in such cases.

This report is based on the results of over one hundred experiments performed on the same individual, a normal adult male. The Grollman acetylene method was used throughout and the experiments were performed under basal conditions. Adequate precautions were taken to prevent the erroneous high outputs in the recumbent position previously reported by Grollman. The length of time for standing was varied from eight to sixteen minutes with no significant change in output. The experiments on the recumbent and standing positions were performed immediately on awakening. This may explain the extreme reductions with standing. Those in the sitting position were done about one hour after rising but under basal conditions. The following table summarises the results obtained.

POSITION	O	AVD	C.O.	C.I.	C.I. DEVIATIONS			P.R.
					Max.	Min.	Av.	
Recumbent (21 expts.).....	206.5	59.97	3.45	2.10	0.296	0.234	0.139	50
Sitting (56 expts.).....	200.7	60.91	3.31	1.99	0.394	0.525	0.134	52
Standing (24 expts.).....	227.9	90.38	2.54	1.53	0.368	0.290	0.120	65

The results in the case of the recumbent and sitting positions agree with Grollman. The decrease with standing is in agreement with recent reports by Donal, Gamble and Shaw, and Schneider and Crampton.

The validity of the Grollman method for cardiac outputs above the basal value.

J. C. SCOTT (by invitation), H. C. BAZETT, M. E. MAXFIELD (by invitation) and M. D. BLITHE (by invitation). Departments of Physiology, University of Pennsylvania and Hahnemann Medical College. (Read by title.)

Grollman's contention that the times at which samples are taken are unimportant under basal condition if 26 feet is not exceeded is confirmed, and also his claim that the method is not valid for high cardiac outputs. Under certain conditions fictitious cardiac indices may be calculated which are low, with no indication that the critical time has been exceeded, when actually they are much higher. This is particularly true if a procedure reduces the true AV difference, so that the apparent higher AV difference calculated as the result of recirculation, is not inherently improbable. Such errors are greater the higher the cardiac index, as they should be theoretically, and the error due to decreased acetylene absorption may be partially, completely or excessively compensated in an unpredictable manner by diminished oxygen absorption, if the oxygen tension falls too low. Examples of the apparent differences and cardiac indices calculated from comparison of samples taken *a*, at 12 to 15; *b*, 17 to 20, *c*, 23 to 26 and *d*, 30 to 35 seconds are appended. The indices calculated from *a* and *b* are considered as valid, and usually as unaffected by mixing errors. The errors of the other pairs are greater the higher the cardiac index, and an index may be calculated from *b* and *c* that gives no indication that the time intervals used were dangerously long.

SUBJECT	CONDITION	A AND B		B AND C		C AND D	
		AV	CI	AV	CI	AV	CI
1	Basal-sitting	71.9	1.6	76.8	1.5	79.9	1.45
	Meal	52.9	2.7	84.8	1.70	70.9	2.0
	Pyrexia	50.3	2.7	69.2	1.95		
		58.8	2.5	60.0	2.45		
2	Basal-sitting	58.1	2.2	66.4	1.9	69.3	1.8
	Meal	51.3	2.95	63.2	2.4	63.9	2.35
3	Basal-sitting	60.5	2.0	65.0	1.85	66.0	1.8
	Meal	59.2	2.55	52.6	2.85	53.3	2.8
	(Basal)	45.8	2.5	62.7	1.85		
	Pyrexia	66.6	2.3	68.4	2.25		
4	Basal-sitting	68.8	1.75	69.9	1.7	66.3	1.8
	Meal	60.9	2.5	75.5	2.1	80.0	1.9
	Pyrexia	52.5	2.65	50.9	2.7	61.0	2.25
		61.3	2.9	74.3	2.35		
		56.5	2.55	68.0	2.1		
		82.5	1.9	75.4	2.1		

Studies on pancreatic grafts made with new technique. W. A. SELLE (introduced by F. C. Mann). Department of Physiology, University of Texas Medical School, Galveston. (Read by title.)

Using the tissue culture technique recently described by Stone, Owings and Gey for transplanting thyroid and parathyroid glands, attempts were made to reduce the severity of experimental diabetes by homeo-transplantation of cultured pancreatic tissue. Small fragments of pancreas, containing mostly islet tissue obtained from fetal animals and animals in which the pancreatic ducts had been ligated ten weeks previously, were cultured for four to seven days on a medium composed of Tyrode's solution, beef extract and the serum of the future host. From thirty to fifty cultured fragments, 1 to 2 mm. in diameter, were implanted subcutaneously in pockets prepared near large blood vessels in the region of the groin and axilla. Cultures from fetal pancreas were implanted in four totally depancreatized dogs; cultures from duct-ligated pancreas were implanted in five totally depancreatized and two sub-totally depancreatized animals. The grafts did not grow in any of the animals, and were completely absorbed after three weeks. It is doubtful if the grafts were at any time functionally active.

Endocrine interrelations during gestation. HANS SELYE, THOMAS McKEOWN (by invitation) and CHARLES HARLOW (by invitation). Department of Biochemistry and Pathological Chemistry, McGill University.

Ovariectomy during gestation does not necessarily interfere with the life of the placenta in the rat. It terminates pregnancy only because it causes the death of the fetus by pressure due to the partial involution of the uterus. The dead fetus, if it is sufficiently large, will cause the abortion of the whole gestation sac, simply acting as a foreign body.

The placenta of the rat produces a hormone similar to or identical with the corpus luteum hormone, since the uterus shows pregestational changes, and the mammary gland develops fully and even lactates as long as nine days after the simultaneous removal of the ovaries and all embryos.

The effect of oestrin on the vagina of the rat is inhibited by the placenta, even after ovariectomy.

In hypophysecomized and ovariectomized pregnant rats the mammary gland is maintained in a well developed condition by the surviving placenta, but it does not secrete milk.

We must consider the first part of gestation in the rat as superimposed upon a pseudo-pregnancy. During this time, removal of the embryos and their placentae does not interfere with the maintenance of the corpus luteum of gestation and the dioestric condition. During the second half of gestation, however, removal of the placentae is shortly followed by the reappearance of oestrous cycles.

The presence of mature placentae, or even of a large deciduoma, in one horn of the uterus interferes with the formation of deciduomata after traumatic injury in the other horn, even though active corpora lutea be present in the ovary.

The renal excretion of creatinine in man. JAMES A. SHANNON. Department of Physiology, New York University, University and Bellevue Hospital Medical College.

When the creatinine concentration of the plasma is raised the creatinine clearance in man is depressed, both absolutely and relative to the simultaneous inulin clearance. At plasma levels of 5 to 15 mgm. per cent the

creatinine clearance exceeds the inulin clearance by an average of 40 per cent, while at plasma levels of 100 mgm. per cent of creatinine the clearance of this substance exceeds the inulin clearance by only 10 per cent. This relationship is believed to be independent evidence of the secretion of creatinine by the renal tubules in man.

It is suggested that the level of glomerular filtration lies between the lowest creatinine clearance obtained at high plasma levels of this substance and the inulin clearance; and in view of the fact that at the highest plasma levels observed the creatinine clearance is still presumably elevated by secretion, it is probable that the level of glomerular filtration lies closer to the inulin clearance than to the creatinine clearance. This is believed to be independent evidence against any extensive active reabsorption of inulin.

Phlorizin administered in doses of 100 mgm. per kilogram brings the clearances of creatinine and inulin together, presumably by a specific depressant action on the tubular secretion of the former substance. The identity of these clearances under phlorizin is evidence against significant passive diffusion of creatinine under the conditions of the experiment.

The origin of protein in the urine of albino rats. H. E. SHIH (introduced by T. Addis). Stanford University School of Medicine.

The urine of all rats contains no inconsiderable amounts of protein, but in ♂ rats at about the age of puberty this proteinuria increases until at 90 days of age it is three or four times greater. In adults ♂ this high level decreases somewhat as age advances but remains always greater than in ♀ rats. This phenomenon has been already described and the following data in general confirm the previous observations.

TABLE I
Rates of protein excretion per 12 hours per 100 sq. cm. body surface

AGE <i>days</i>	♂ RAT <i>mgm.</i>	♀ RAT <i>mgm.</i>	CASTRATED ♂ <i>mgm.</i>
40	0.4	0.3	0.3
50	0.5	0.3	0.3
60	0.9	0.3	0.2
70	1.2	0.2	0.4
80	1.3	0.1	0.5
90	1.4	0.2	0.8
100	1.0	0.1	0.3
110	0.8	0.1	0.6
120	0.9	0.2	0.3
130	1.1	0.2	0.4
140	1.0	0.2	
150	1.0	0.1	0.2

It has also been observed that castration before puberty reduces but does not entirely obliterate the difference between ♂ and ♀. This effect is also seen in the results shown in table 1.

The following experiment indicates that in the ♀ the urinary protein originates from the kidney but that in the ♂ part is derived from secretions of the glands of the genital system. Rats of from 90 to 110 days of age were placed under pentobarbital anesthesia and both ureters ligated. The

bladder was punctured at its most avascular region with a fine needle and irrigated with 1 to 3 cc. amounts of 0.9 per cent NaCl, for a period of 2 hours, and the protein measured. Table 2 shows that while no protein was found in the washings from the ♀ it reappeared in those from the ♂ rats. The result suggests that the high rate of protein excretion in the ♂ is due to addition of extra-renal protein. The extra-renal source of the additional protein in the ♂ was proved by the results of experiments in which all the accessory sex glands were excised.

TABLE 2

Rate of protein excretion in mgm. per 12 hours per 100 sq. cm. of body surface after ligation of both ureters

MALE		FEMALE	
Number	Protein excretion	Number	Protein excretion
1	2.65	1	0
2	0.66	2	0
3	0.66	3	0
4	1.17	4	0
		5	0

The effect of motion on the electrical conductivity of blood. EDWARD SIGMAN (introduced by Louis N. Katz). From the Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital and the Department of Physiology, University of Chicago.

The electrical conductivity of blood increases when the blood is put in motion. This was measured with a 1000 cycle oscillator and a standard bridge circuit. The graph relating the electrical conductivity to the rate of blood flow forms a smooth curve, convex upward. Various colloidal and electrolytic solutions failed to show this phenomenon. The change in electrical conductivity is dependent upon the presence of erythrocytes, since this phenomenon disappears when the blood is laked. In centrifuged specimens the change in electrical conductivity with flow is found only with that portion containing the erythrocytes. The pattern of the change in electrical conductivity alters when the concentration of the erythrocytes is changed. Dilution of the erythrocytes with saline gives a less marked difference in electrical conductivity than dilution with serum.

The absorption of water, chlorides and glucose by the adrenalectomized rat. HERBERT SILVETTE. Physiological Laboratory of the University of Virginia Medical School. (Read by title.)

Both normal and adrenalectomized white rats injected intraperitoneally with 5 per cent glucose plus 1 per cent sodium chloride solution showed at the end of the experimental period of 4.5 hours almost complete (over 90 per cent) absorption of sugar. The normal animals absorbed on the average 60 per cent and the adrenalless animals 70 per cent of the injected chlorides. Practically no water was taken up by the normal animals, while the operated animals absorbed over 20 per cent of the total amount administered.

A series of normal rats given intraperitoneal injections of 2 per cent sodium chloride absorbed by the end of the observation period (6 hours)

10 per cent of the water and 80 per cent of the chlorides. In the same time the adrenalectomized rats absorbed 45 and 90 per cent respectively. The absorbed chloride was found in the greatest concentration in the blood stream, and in a lesser degree in the muscle and liver. The greatest water storage and percentage increase took place in the muscles. The adrenalectomized white rat showing early symptoms of insufficiency is therefore able to draw large amounts of injected salt and water into its blood stream. In so doing it demonstrates in striking manner the need of the adrenalless organism for these materials.

Absorbed glucose in the normal rats was stored mainly in the liver as glycogen. In the adrenalectomized animal the absorbed glucose was not converted to any noteworthy extent into liver or muscle glycogen. Apparently such absorbed glucose was held in the blood-stream—the hyperglycemia being much more marked in the adrenalectomized (glucose-injected) animals—and probably largely in the tissues by simple inundation. Twenty animals were used in the study. Further experiments are in progress.

Salt loss following adrenalectomy and glucose administration. HERBERT SILVETTE. Physiological Laboratory of the University of Virginia Medical School. (Read by title.)

Following adrenal removal in cats, serum sodium and chloride concentrations fell on the average to levels 11 and 9 per cent respectively below the normal. Muscle and liver sodium and chloride values were also reduced, while the water content of these tissues was increased. Although there was some indication that the serum protein content was slightly reduced after adrenalectomy, there appeared to be no correlation between the reduction and the increased water concentration in the muscles of cats suffering from adrenal insufficiency.

Intraperitoneal injections of glucose solutions reduced the serum and tissue sodium and chloride concentrations below those observed in adrenal insufficiency. In spite of the salt loss observed in these experiments, however, glucose-injected normal cats remained in apparently good health unless the serum sodium and chloride levels were reduced 26 and 33 per cent respectively below the normal values.

Sodium chloride balance and carbohydrate metabolism in the adrenalectomized opossum. H. SILVETTE and S. W. BRITTON. Physiological Laboratory of the University of Virginia Medical School.

After adrenal removal the opossum (*Didelphys virginiana*) showed marked increases in sodium and chloride concentrations in the blood serum. The average increase in serum sodium was 10 per cent, and in serum chloride 13 per cent over the normal levels. This was in striking contrast to the decreases found after adrenalectomy in more recent phylogenetic species, e.g., cat and dog. Muscle sodium was also definitely increased (25 per cent), although the chloride levels in the muscle were apparently unchanged. Urinary excretion of sodium and chlorides was concomitantly decreased after excision of the glands, thus explaining in part the retention of sodium chloride by the adrenalless organism.

Carbohydrate reductions were noted in all the tissues examined. Serum sugar fell 35 per cent below the normal average; liver glycogen 75 per cent, and muscle glycogen 60 per cent below values observed in normal animals.

The adrenalectomized opossum apparently dies from carbohydrate depletion, and not from any disturbance in its sodium chloride balance. The theory that sodium chloride is controlled by the adrenal cortex is not supported on the basis of the present results.

The changes in salt balance in the adrenalectomized opossum, so reversely different from those observed in the case of adrenalectomized rats, cats, dogs and guinea pigs, may be correlated with the histological picture of unspecialized convoluted tubular epithelium and atypical glomerular structure reported by MacNider (*Proc. Soc. Exp. Biol. Med.* **25**: 130, 1927) in the opossum kidney. Possibly one of the effects of the life-maintaining hormone, or the effect of perhaps another discrete but still undetermined hormone, may be the maintenance of normal kidney function. This possibility and the involved mechanisms are now being investigated.

Salivary secretion during thirst. ERMA A. SMITH. Iowa State College, Ames, Iowa.

Salivary secretion during a control interval was measured in a series of normal adults. Thirty to 100 cc. saturated solution of sodium chloride was then introduced into the stomach and the collection continued during the succeeding period of desire for water. The flow of saliva was diminished in some subjects; in others it was increased; in none did it cease. Subjectively the call for water was assigned to the throat or stomach.

The excretion of inulin, xylose and urea by normal and phlorizinized man.

HOMER W. SMITH and JAMES A. SHANNON. Department of Physiology, New York University, University and Bellevue Hospital Medical College.

The excretion of the polysaccharide inulin (the molecular weight of which is variously given at 975 to 4860) has been examined in man after intravenous infusion, and the inulin clearance has been compared with simultaneous urea, glucose and xylose clearances in both normal and phlorizinized man.

The inulin clearance in normal man is independent of the plasma concentration, and the curves relating plasma concentration to quantity excreted per unit time extrapolate to zero coordinates. This fact, combined with the fact that inulin is not secreted by the aglomerular fish, and with other evidence, indicates that the mammalian kidney cannot secrete carbohydrates in general, and supports the tentative conclusion that inulin is not secreted by the renal tubules in man. The inulin clearance exceeds the simultaneous xylose clearance in normal man by an average of 22 per cent.

In the light of this evidence it appears that some xylose (and sucrose) is normally reabsorbed by the renal tubules from the glomerular filtrate; this reabsorption is in part an active process, since the disparity between the inulin and xylose clearances is in part removed by phlorizin; that part of the disparity (10 per cent) which is not removed by phlorizin may be due to passive diffusion of the xylose from the tubular urine back into the blood or lymph. It seems scarcely probable that in the normal kidney there is any significant diffusion of inulin, even accepting a molecular weight as low as 975; but in view of the fact that evidence has been uncovered for the active reabsorption of xylose, evidence against the active

reabsorption of inulin should be obtained before the inulin clearance is accepted as a measure of glomerular filtration.

The relation between lactic acid concentration and oxygen consumption in resting and active muscle. PAUL W. SMITH. Department of Physiology, College of Medicine, University of Illinois, Chicago.

Sartorius muscles of small frogs were measured *in situ* and placed in a Thunberg-Fenn apparatus so modified that changes could be made in the length of the muscle without removing it from the chamber. Resting metabolism and the excess due to 10 break induced current stimuli were measured at various fiber lengths. Both resting and extra or activity oxygen consumption increased progressively with increase in fiber length up to approximately 120 per cent of the *in situ* length; upon further increase in length the extra oxygen consumption due to activity decreased in accordance with the results of Evans and Hill (J. Physiol. **49**: 10, 1914), on the activity heat production, while the resting oxygen consumption rose more sharply. The total oxygen consumption, resting plus activity, increased progressively up to the maximum degree of extension, in agreement with Starling and Visscher (J. Physiol. **62**: 243, 1927), and others, on the total metabolism of the heart.

Sartorii of bull frogs were measured *in situ*, removed, suspended in a moist chamber, and after 5 minutes in an atmosphere of nitrogen were given 120 maximal break induced current stimuli. One muscle of a pair was stimulated either at its *in situ* length or at 5 or 10 per cent increased length. Its mate was stimulated at 10, 20, or 25 per cent increase over the *in situ* length. One hundred twenty stimuli, in the case of the longer muscle, produced more lactic acid than in its shorter mate, until approximately 20 per cent increase in length had been reached. Beyond this point the longer muscle shows a lower lactic acid content than its mate, even though the latter be stimulated at or near its *in situ* length. This is in agreement, not with the total, but with the extra oxygen consumption due to activity. Control muscles kept under stretch, but not stimulated, for the duration of the experiment (7 minutes) show lactic acid values which vary from unstretched controls within the limits of experimental error, although muscles kept in nitrogen under severe stretch for as long as 2 to 3 hours show a lactic acid content near the fatigue level. It is concluded that that portion of the total oxygen consumption at extreme extension representing the resting metabolism is not determined by the concentration of lactic acid in the muscle, for if this were the case lactic acid accumulation in anaerobiosis should correspond to the total rather than the extra oxygen requirement.

The rôle of the anterior pituitary gland in pancreatic diabetes and diabetes mellitus. SAMUEL SOSKIN, I. ARTHUR MIRSKY (by invitation), L. M. ZIMMERMAN (by invitation) and N. CROHN (by invitation). The Metabolic Laboratory, Department of Physiology, Michael Reese Hospital and the Department of Physiology, University of Chicago.

Since the 1930 report of Houssay and associates on the alleviation of the diabetic syndrome in the hypophysectomized-depancreatized dog, we have studied 8 such animals. Our results are in agreement with those of Houssay, but not with those of the subsequent investigators who reported complete disappearance of the diabetes.

Our animals survived without insulin for varying periods up to 4 months. In spite of their apparent well-being, marked hyperglycemia and glycosuria were constantly present in those animals which ate their diet, and all animals eventually lost weight in spite of the adequate diet offered them. However, when the animals ate very little or when food was withheld, an immediate and rapid decrease in the blood sugar level and the glycosuria always occurred. The blood sugar in these instances sometimes fell to hypoglycemic levels and the urine was sugar free.

Another striking difference from the depancreatized dog was the absence of the ketone bodies from the urine of our animals regardless of the degree of hyperglycemia and glycosuria, or the length of survival without insulin after pancreatectomy.

We have applied the observations of Barnes, Reagan and Nelson and of Nelson and Overholser as to the inhibitory effect of oestrogenic hormone on the anterior pituitary, to patients with diabetes mellitus. The results of this work are essentially the same as those obtained with the dogs, in that the well-being of the patient could be maintained when insulin was entirely withdrawn and there was some temporary alleviation of the hyperglycemia and glycosuria. However, as in the animals, no ketone bodies appeared in the urine throughout the period of observation, regardless of the degree of the carbohydrate manifestations.

Our results are in agreement with those of Anselmino and Hoffman as to the importance of the anterior pituitary gland in fat metabolism. The absence of the ketone bodies, the hypoglycemic effect of starvation and the uniformly low D:N rations we have obtained, indicate that fat is the source of a portion of the sugar which appears in the blood and urine of the diabetic organism, and that the anterior pituitary gland exerts an important influence on this conversion.

The influence of variations of O₂ and CO₂ tension in the inspired air upon cortical and subcortical processes in men. IRWIN G. SPIESMAN (by invitation) and E. GELLHORN. Department of Physiology, College of Medicine, University of Illinois, Chicago.

1. The influence of O₂-lack and CO₂ on cortical processes was studied by determining the acoustic threshold with the aid of an audiometer and by measuring the duration of the latent period of negative after-images under standard conditions. All experiments were carried out on thoroughly trained subjects. The results are:

O₂-lack produced by breathing air from a Douglas bag containing 7.5 to 11 per cent for 7 to 30 minutes increases the hearing threshold and lengthens the latent period of negative after-images. The effects are considerable and may extend over a long time in spite of the readmission of air or of even richer O₂-mixtures. The effects of inhalation of 4 to 7 per cent CO₂ are similar but less severe. The recovery is faster than with O₂-lack. Maximal voluntary hyperpnea carried out for 2 to 6 minutes at a rate of 35 to 90 p.m., also increases hearing threshold and lengthens the latent period of negative after-images. The effects are observed not only during the apnea but also for some time afterwards when the respiration has become normal. Control experiments indicate that the effects are not due to vasomotor or blood pressure changes, nor can they be explained by psychic changes (attention).

2. Similar experiments performed in order to study the effect of these

factors on caloric nystagmus indicate qualitative differences in the excitability of brain stem and cortex. O₂-lack may be of no influence on nystagmus under conditions which regularly influence auditory and visual processes. Further extension of the O₂-lack period leads to a decrease in nystagmus. CO₂-inhalation and hyperpnea exert opposite effects on nystagmus, the former causing a decrease, the latter an increase. There are no after effects.

According to these investigations it seems to be characteristic of the sensory cortex that it reacts to any alteration in the O₂ and CO₂-tension of the blood with a decrease in excitability and relatively long after effects. These changes in excitability are most likely due to a cellular disturbance in the visual and auditory cortex. In contradistinction to these findings, the brain stem mechanism involved in nystagmus reacts (qualitatively and quantitatively in a different manner) from the cortex, but similarly to the spinal cord.

A method for recording volume changes of the large intestine in normal dogs, with special reference to cathartics. F. R. STEGGERDA and C. C. GIANTURCO (by invitation). Department of Physiology, University of Illinois, Urbana.

By operation, very small lead shots were inserted with a specially devised instrument just underneath the serous coat of the cecum and colon of dogs. They were placed about one-half inch apart, so that the entire colon was outlined to within an inch of the rectum. Our purpose was to study the size of the empty colon by means of x-ray, by eliminating the barium meal, and to learn the effects of the cathartics, magnesium sulphate and castor oil, on the volume changes of the cecum and colon in the normal unanesthetized animal.

Each experiment was carried out on animals fasted for a day, in the following manner: after taking a control x-ray picture of the colon, we administered by stomach tube 5 grams of magnesium sulphate in 100 cc. of water, or 1 ounce of castor oil. Thereupon we took pictures at 15 to 30 minute intervals until defecation occurred, after which the final picture was taken. The cross-sectional area of the colon in each plate was determined in square inches by means of a planimeter, and plotted against time.

In the case of magnesium sulphate the colon increased in size about 100 per cent before defecation, reaching its maximal distention in 2 to 3 hours. After defecation the colon returned to its original size. Castor oil caused defecation more frequently with a distention of less than 50 per cent.

Conditions determining local graded contractions in the skeletal muscle fiber. S. E. STEIMAN (introduced by F. H. Pratt). Department of Physiology, Boston University School of Medicine. (Read by title.)

Contractions were studied in the single fibers of the membrana basihyoidea of *Rana pipiens*. It was found that a fresh preparation with the circulation intact gave only all-or-none responses even when micro-electrodes of 0.5 to 5 μ diameter were employed. But the following were some of the conditions found to be favorable for the production of graded contractions:

1. Absence of circulation in the capillaries supplying the musculature.
2. Injury to the muscle fibers.
3. A preparation that is at least three hours old.

The fibers of a fresh preparation which have been responding in an all-or-none manner will give local contractions if the Ringer solution is modified by addition of 0.028 g. KCl per 100 cc. Graded contractions produced in this way, as well as those obtained from fibers of an old preparation, can be immediately eliminated (with reversion to the all-or-none type) by changing the solution for Ringer plus 0.33 g. NaCl per 100 cc. This effect is reversible at will.

A fresh muscle fiber which is fatigued will readily give graded responses. Frequency of stimulation is another factor in the production of such contractions in fresh uninjured fibers. A stimulation of 30 per minute with an electrode of only $2.5\ \mu$ diameter gave responses of an all-or-none character even after two hours of continuous stimulation; but a frequency of 33 stimuli per minute soon caused graded contractions. The time necessary for the appearance of the local responses shortened with the age of the preparation and with the increase in frequency of stimulation above the minimum of 33 per minute. The addition of 0.33 g. NaCl per 100 cc. of Ringer does not eliminate the local contractions obtained from a fatigued or injured muscle fiber.

When a fiber is capable of giving local contractions, these responses can be obtained by stimulating the fiber with a coarse electrode. With an electrode of 0.1 mm. diameter, the magnitude of the local contractions was observed to diminish with increase in distance between the electrode and the muscle fiber.

The effect of bile acids on the oxygen consumption of dog tissues. WILLIAM H. STRAIN (by invitation) and M. ELIZABETH MARSH. Department of Biochemistry, School of Medicine and Dentistry, and Department of Vital Economics, University of Rochester, Rochester, N. Y.

The effect of varying concentrations of the sodium salts of the bile acids, cholie, glycocholic and taurocholic, on the oxygen consumption of liver, kidney and spleen of the dog was studied by means of the Warburg apparatus. Simultaneous determinations in triplicate on tissue slices were made at 37.5°C. using air rather than pure oxygen. The increase or decrease in oxygen consumption produced by the bile salts dissolved in Ringer's-glucose-phosphate solution was compared with control values where no bile salts had been added. With concentrations expressed in millimols the results may be summarized by giving the concentrations at which definite depression in oxygen consumption was first noted.

Sodium cholate ($M = 430$): Spleen—marked decrease at 0.5–1.0 mM; liver—marked decrease at 1.0 mM; kidney—definite decrease at 1.0–2.0 mM.

Sodium glycocholate ($M = 487$): Spleen and liver show a small but definite decrease at 2.0 mM, while at this concentration kidney is slightly stimulated.

Sodium taurocholate ($M = 537$): Spleen and liver—definite depression only at concentrations above 5 mM; kidney—depression at 8–10 mM, below this concentration a very slight stimulation.

Using decrease in oxygen consumption as a measure of toxicity sodium glycocholate is 3 to 5 times less toxic than sodium cholate while sodium taurocholate is 3 to 5 times less toxic than glycocholate. The differences in toxicity illustrate the advantage of conjugation in detoxication and especially the advantage resulting through conjugation with taurine.

Since only taurocholic acid has been found in the bile of the dog it may be argued that the selectivity exhibited may be a reflection of tissue needs.

Studies on changes with increasing age in the phosphorus fractions of various tissues of the rat. HAROLD CARL STRUCK (by invitation) and MAURICE B. VISSCHER. Department of Physiology, College of Medicine, University of Illinois, Chicago.

It has been found that there is a marked difference in the creatine phosphate, ortho-phosphate, and total acid soluble phosphate fractions of striated muscle, liver, and cardiac muscle of two groups of rats, one approximately three years of age, the other approximately sixteen months old.

The methods used were those of Eggleton and Eggleton (J. Physiol. **68**: 193, 1929) and the results show that tissues of the young mature rats (sixteen months old) contained significantly more of each of the phosphorus fractions analyzed for (creatinine phosphate, orthophosphate, and total acid-soluble phosphate) than did the corresponding tissues of animals approximately three years old. The average total acid soluble phosphorus in the striated muscle in the former was 139 mgm. per cent, and in the latter 81 mgm. per cent. Similar changes occurred in the other tissues.

This alteration in important constituents in the musculature is believed to be of interest in connection with the physiological problem of senescence. These substances are unquestionably important in bodily activity and their decrease with age may be associated with the degenerative changes in senility.

The results of this preliminary study are being confirmed in a more extensive program now under way. Pearson (J. Biol. Chem. **106**: 1, 1934) has reported a decrease in the acid soluble phosphorus in the plasma of the horse with increasing age up to maturity, and Hatai (J. Comp. Neurol. **28**: 361, 1917) has found a decrease in total phosphorus in the brain of rats with increasing age. Similar results were obtained by Koch and Koch (J. Biol. Chem. **15**: 423, 1913).

Potential differences across frog's skin as an index to its structure. MARGARET SUMWALT. Departments of Physiology, University of Pennsylvania and Johns Hopkins University.

Frog's skin gives rise to electromotive forces different from those which could be measured across a relatively simple artificial membrane under like conditions. Some of its diverse electrical peculiarities have received explanations equally diverse; many remain unexplained.

In the present instance, frog's skin has been studied in solutions of varied pH and salt composition. Not only the sign and magnitude of the potential differences have been recorded, but their reproducibility, and the direction in which they tended to drift with time. Potentials in stagnant media were compared with those in flowing solutions. Systematic analysis of the results suggests that the laminated structure of the skin may be largely responsible for its complex electrical behavior, while the individual layers behave simply. The relative electrolyte concentration, and ion, permeability of the more superficial layers may be deduced tentatively on the basis of the results. The schematic picture of skin structure obtained in this way explains many of the electrical peculiarities already known, which have hitherto received diverse explanations.

The influence of diet on the survival of adrenalectomized rats. H. G. SWANN (introduced by B. O. Barnes). Department of Physiology, University of Chicago.

Many conflicting reports have been made concerning the mortality of rats after adrenalectomy. It seemed possible that dietary effects might be involved. Accordingly, the mortality after adrenalectomy of rats on various balanced diets was studied, 28 day old animals being employed. The diets tested included types high in carbohydrate, fat, protein, salts, and milk. In the experiment, approximately 770 rats were used, 620 being from one colony.

It was found that all groups on a diet containing bread with fresh milk averaged a mortality of 55 per cent at 12 days after operation and 71 per cent at 21 days after operation. All groups lacking this combination averaged a mortality of 90 per cent at 12 days and 95 per cent at 21 days after operation. One hundred and fifty rats from three other colonies were also tested and reacted similarly.

Mature rats are, in general, subject to the same conditions. They differ from young ones, however, in that their mortality rate is less rapid and in that the mortality on the bread and milk diet is low. At 21 days after adrenalectomy, 15 per cent of those on the milk combination had died whereas 90 per cent of those lacking this combination had died. These experiments suggest that under our conditions diet plays an important rôle in the survival of adrenalectomized rats.

Ergotoxin and rage. N. B. TAYLOR, C. B. WELD (by invitation) and J. F. SYKES (by invitation). Department of Physiology, University of Toronto.

The administration of ergotoxin phosphate intravenously to cats (0.5 mgm. per kgm.) produces with great regularity a condition of apparent rage, simulating closely the "sham rage" described by Cannon and by Bard. The reaction is "central" and is probably caused by functional removal of cortical inhibition from the rage centre, as evidenced by the following points:

1. Rage, limited to the head region, is still obtained by ergotoxin after sectioning the cervical cord.
2. Adrenalectomy does not abolish the response.
3. A conditioned reflex (salivary) is either abolished or much reduced.
4. No striking change in body temperature occurs.
5. Toxic doses of parathormone or irradiated ergosterol known to cause exaggeration of central inhibitory processes, do not abolish the rage response.
6. Decerebrate rigidity may be abolished, and a transient loss of extensor tone often follows ergotoxin administration.
7. Dogs fail to show rage but exhibit these postural changes to a marked degree.

The effect of coronary occlusion on myocardial contraction. ROBERT TENNANT (introduced by C. J. Wiggers). Department of Physiology, Western Reserve University Medical School, Cleveland, Ohio.

A small optical transmission myograph with limbs 1.5 cm. apart and with the lever arm pivoted in jewelled bearings registers reasonably satis-

factory curves of muscle shortening when securely stitched to the surface of the left ventricle in the longitudinal direction of the muscle fibers.

The normal myograms show *a*, several small vibrations during the isometric contraction phase; *b*, a sharp rise synchronous with the elevation of aortic pressure; *c*, a continued plateau which either persists until the end of isometric relaxation or exhibits a second small elevation immediately following the incisura, and *d*, an abrupt fall coincident with ventricular filling from the auricles.

The small oscillations of isometric contraction and possibly the sustained plateau or further rise during isometric relaxation may represent artefacts superimposed on the contraction curve. Regardless of whether the latter is an artefact or not the curves demonstrate that lengthening of the muscle fibers does not occur until ventricular filling commences.

After ligating the anterior descending branch of the left coronary artery a progressive decrease in the degree of systolic shortening occurred. Somewhat later the contractions appeared to end earlier and earlier in systole and an actual lengthening of the fibers by the force of the high intraventricular pressure resulted. At a mean interval of only 59 seconds after ligation, the curve showed, characteristically, a sharp deep fall below the diastasis level during isometric contraction and a maintenance of this minimum until the end of isotonic contraction. This was followed by a sharp rise to the diastolic level precisely during isometric relaxation with a subsequent gradual decline to a diastasis plateau. This form of curve persisted until circulation was restored to the part or ventricular fibrillation terminated the experiment. With restoration of circulation a gradual return to the normal curve was observed up to 23 minutes of ischemia.

Obviously, ischemia of one minute duration either abolishes the contractile power of the affected muscle or reduces it to the extent that it offers no resistance to intraventricular pressure.

The effect of sympathectomy on the vasomotor carotid sinus reflexes of the cat.

CAROLINE BEDELL THOMAS (by invitation) and CHANDLER M. BROOKS.

Department of Physiology, Johns Hopkins School of Medicine.

The thoracic and abdominal sympathetic chains of eleven cats were completely removed in two stages thus severing all connections between the spinal cord and postganglionic sympathetic fibers. Two or three weeks later, under nembutal, chloralose or light ether anesthesia, the animals were subjected to the following procedures: clamping and unclamping the common carotids, perfusion of the isolated carotid sinuses with Ringer's solution at different pressures, and stimulation of the sinus nerve. At the start of each experiment the vago-depressor nerves were cut bilaterally to denervate the heart and to eliminate reflexes originating from the aortic zone. Arterial pressure was recorded by a mercury manometer connected with one femoral artery. At autopsy each cat was carefully examined to make sure of the completeness of the sympathectomy.

In the sympathectomized cats no reflex change in arterial pressure was ever obtained by raising or lowering the pressure within one or both carotid sinuses. On the other hand, when these procedures were carried out under the same conditions in sixteen normal animals, marked depressor and pressor responses were invariably elicited. Small changes in arterial pressure (never greater than 15 mm. of mercury) occurred on clamping and unclamping the common carotids in the sympathectomized cats.

The mechanical, non-reflex nature of these was indicated by their persistence after denervation of the sinuses or cervical transection of the cord and by their occurrence on occluding and opening the external carotids. After sympathectomy, faradic stimulation of a sinus nerve with currents of moderate intensity had no effect on arterial pressure, and only when the current was so strong that marked respiratory changes and muscular activity appeared was a fall in blood pressure of 5 to 30 mm. noted. It is concluded that removal of the sympathetic chains interrupts all the physiologically important efferent paths of the vasomotor reflexes evoked by changes in pressure within the carotid sinus.

The effect on the pyloric sphincter of pressure changes in the duodenum.

J. EARL THOMAS and J. O. CRIDER (by invitation). The Department of Physiology, Jefferson Medical College, Philadelphia.

In experiments on dogs with permanent gastric and duodenal fistulae, water under measured pressure was allowed to flow through a tube into the duodenum near the pylorus. Escape of water was prevented by means of a balloon which encircled a perforated portion of the duodenal tube and was, therefore, constantly inflated with water at the experimental pressure. The reaction of the pyloric sphincter was recorded by the pressure tonometer (this Journal 88: 498) or inferred from measurements of the rate of flow of water through the pylorus into the stomach.

Pressures up to 20 cm. of water did not ordinarily cause a maintained increase in pyloric tonus; pressures of 30 cm. or more either caused pylorospasm and vomiting or, less frequently, a marked relaxation of the pylorus with or without vomiting. The more detailed results obtained with the pressure tonometer show that pressures between 10 and 20 cm. of water, when first applied, caused one or more active contractions, frequently associated with a temporary increase in tone of the sphincter, generally followed by a return to normal if the pressure remained constant. Preliminary measurements indicate that the normal pressure within the duodenum seldom rises above 10 cm. of water except momentarily; consequently the observed reactions of the sphincter appear to be adequate to control regurgitation of duodenal contents into the stomach in all ordinary circumstances.

Urine formation under anoxia in dogs. L. A. TOTH (introduced by E. F. Adolph). Department of Physiology, School of Medicine and Dentistry, The University of Rochester.

The breathing of uniform, low oxygen tensions varying from 4.8 to 8.9 per cent for periods of 12 to 122 minutes resulted in a marked diminution in the rate of urine excretion. This was true in 16 of 20 experiments on 12 dogs, lying on their backs, anesthetized (by stomach) with diallyl-barbituric acid plus urethane (Dial). Of the remaining 4 experiments 2 gave a diuresis followed by an oliguria, while 2 experiments showed no change from the control excretion rate. The presence of CO₂ up to 4.8 per cent did not change the urine responses.

Of the 16 oliguria experiments, 6 showed recovery to the control level in the urine excretion with the readministration of room air, while 10 showed a marked temporary diuresis.

The arterial blood was approximately 31 per cent saturated with oxygen when 5.8 per cent oxygen was breathed. The plasma concentrations

did not change significantly during the anoxia. There was no constant relationship between rate of urine excretion and carotid blood pressure. The chloride and urea concentrations in the urine decreased in most cases; the rates of total chloride and urea excretions were nearly proportional to the water excretion. The chloride concentration remained low during the recovery period but the urea concentration showed a gradual increase to the control level. The above results are not similar to the results in Starling's cyanide experiments.

Possibly the increased secretion of epinephrine resulting from anoxia was one of the factors producing some of the above results. Intravenous injections of epinephrine produced oliguria and diuresis even in the same dog; intramuscular and subcutaneous injections seemed to produce only diuresis. The chloride and urea concentrations and their excretion rates varied in the same way in these experiments as in the anoxia experiments.

In 5 experiments on an unanesthetized dog with a bladder fistula, the dog standing in a stall, breathing of 5.6 to 10 per cent oxygen for periods varying from 65 to 161 minutes produced not oliguria but distinct diuresis. The chloride concentration of the urine increased and the urea concentration decreased during the first part of the anoxia; in the latter part they returned to control levels.

The colloid osmotic pressure of the blood plasma in fishes. ABBY H. TURNER.
Woods Hole Oceanographic Institution and the Physiological Laboratory,
Mount Holyoke College.

Because of the apparent significance of the colloid osmotic pressure of the blood plasma in maintaining a balance between blood and tissues fluid in mammals, an accumulation of data regarding colloid osmotic pressure in lower vertebrates has been begun. Determinations have been made by the method of Krogh and Nakazawa on 101 individual fish representing six species of teleosts and two of elasmobranchs obtained at Woods Hole and two species of trout secured from the Massachusetts State Fish Hatchery at Sunderland, with also a few scattered representatives of other species, marine and fresh water. Refractive indices have been determined, and in the latter part of the study, whenever the size of the samples made it possible, total nitrogens and protein nitrogens. Difficulties lie in our complete ignorance of the previous history of individual fish, save for the trout, and in the dependence of valid results on the use of entirely uninjured specimens.

Summary of results. 1. The range of colloid osmotic pressure for the plasma of each species of teleost is probably characteristic, though the findings for different species overlap. Averages range from 93 to 128 mm. water, with no apparent significant difference between the fresh water and marine species studied.

2. The range of colloid osmotic pressure in elasmobranchs is much lower than in teleosts, with the averages for the two species studied and single determinations on two other species close together, near 38 mm. water.

3. No sex differences have been noted.

4. A group of 3 young brook trout averaged 145 mm. as contrasted with 100 mm. for 12 older specimens, all fish very well fed.

5. Scattering determinations suggest that the range for strong swimming fish may be higher.

6. Total nitrogen and protein nitrogen figures and refractive indices parallel the findings for colloid osmotic pressure only approximately.

Further investigations on the control of infections of the upper respiratory tract and middle ear. R. G. TURNER (introduced by T. L. Patterson). Medical Research Department, Wayne University, College of Medicine, Detroit, Michigan.

The bacteriology of the nasal cavities and middle ear of 115 albino rats is reported. Fifty-two of these animals were killed during the period of maintenance or loss in weight due to lack of vitamin A. Eleven, after 13 days or more of maintenance or loss in weight, received cod liver oil as a curative measure and 25 animals received intranasal injections of a 24 hour broth culture of a non-virulent strain of *Staphylococcus albus* in addition to the cod liver oil. Twenty-seven animals were kept as controls and received cod liver oil from the beginning of the experiment. Five of these were given intranasal injections of the broth culture for a period of 24 days or more.

Animals treated for 21 days or more with cod liver oil and intranasal injections of the broth culture were free from suppurations in the nasal cavities and middle ear. Pathogenic bacteria were not encountered. In the group receiving only cod liver oil as a curative measure middle ear infection was present in 18.1 per cent of the animals.

Levulose and galactose tolerance in Eck fistula dogs. JOHN VANDOLAH (introduced by Lathan A. Crandall, Jr.). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Illinois.

The tolerance of normal and Eck fistula dogs to levulose and galactose was studied by the oral or intravenous administration of these sugars in amounts sufficient to cause urinary excretion of the excess. A figure for the "tolerance" was obtained by subtracting the amount spilled in the urine from the amount given.

The amounts of galactose tolerated are substantially the same for normal and Eck fistula animals with doses of 1.5 to 4.25 grams per kilo, when given orally. For both groups of animals the percentage excreted in the urine increases as the amount given is increased. Normal dogs tolerate up to 8.0 grams of levulose per kilo without spilling more than 1.5 per cent, while those with Eck fistulae spill approximately 10 per cent at doses from 4.0 up to 8.0 grams per kilo. The blood sugar rise (expressed as dextrose) is somewhat higher in Eck fistula dogs than in normals after either galactose or levulose orally. It was noted that the increase in blood sugar in normal animals is greater with galactose than with levulose.

Intravenous injection of these sugars by Woodyatt pump over one hour periods essentially confirms the finding that following the Eck fistula operation the tolerance for levulose is more greatly decreased than that for galactose.

The composition of the genital tract of the macaque at various stages of the menstrual cycle. H. B. VAN DYKE and GRAHAM CH'EN (by invitation).

Department of Pharmacology, Peiping Union Medical College.

Water, total solids, ash and ash constituents, total nitrogen glycogen, and, in some cases, total lipoids were determined in separate samples of mucous membrane and musculature of the corpus uteri, cervix uteri and vagina of macaques at different stages of the menstrual cycle. The attempt was made to study only adult ovulatory cycles coincidently with

menstruation, or when an ovary contained either a ripening follicle or a newly-formed corpus luteum. Data on the effects of ovariectomy with or without subsequent injection of theelin were also secured.

The effect of anoxemia on the absorption of water from the small intestine.

EDWARD J. VAN LIERE, N. A. DAVID (by invitation) and D. H. LOUGH (by invitation). University of West Virginia, Morgantown.

The effect of anoxemia on the absorption of water from the small intestine was studied on barbitalized dogs and cats. Both adult and young animals were used. Two animals were chosen which were as near the same weight and age as it was possible to get them (in some instances littermates were used); one animal served as the control and the other was subjected to anoxemia.

The abdomen was opened in the mid-line and the intestines were exposed. A ligature was placed above the ileocecal valve and another ligature was placed about the jejunum at its upper part, so that with the exception of the duodenum, practically the entire small intestine served as a loop. The intestine was measured so that the loops were of the same length in the two animals. Tap water at body temperature was then introduced into that part of the intestine which had been tied off. Care was taken to avoid undue distention. Attention was given, of course, to all details that were essential for the production of uniform experimental conditions.

Anoxemia was produced by placing the animal in a steel respiratory chamber previously described. Ventilation was adequate so that carbon dioxide could not accumulate. Various degrees of anoxemia were used ranging from about 17 per cent oxygen, which corresponds roughly to an altitude of 5000 feet, to 7 per cent oxygen which corresponds to about 28,000 feet.

The fluid was left in the intestine for 30 minutes. The loop was then removed from the abdominal cavity and the water was carefully measured.

It was found that practically all the animals (about 100 were used) which were subjected to atmospheres which contained 12 per cent oxygen or less, showed more absorption during anoxemia than did the controls. At extreme ranges of anoxemia, such as 7 per cent oxygen, the results obtained with adult animals were indeterminate. The young animals, however, even at this low oxygen level continued to show more absorption than did the controls.

Factors influencing the movement of chloride against its diffusion gradient between intestine and blood. MAURICE B. VISSCHER and RAYMOND C. INGRAHAM (by invitation). Department of Physiology, College of Medicine, University of Illinois, Chicago.

According to classical theories the absorption of inorganic ions from the intestine is a passive process as far as the intestinal epithelium is concerned. It has been supposed that diffusion gradients would account for the flow of such substances from the intestinal lumen to the blood. It has been pointed out by Burns and Visscher (*This Journal* 110: 490, 1934), however, that under certain circumstances, particularly in the presence of sulphate, citrate, or similar ions, the chloride moves into the blood against its diffusion gradient. They showed that the ordinary membrane equilibrium phenomena would not account for this movement of chloride and concluded that the membrane itself intervenes in the movement of the

chloride ion. There is, thus, a secretion-like process involved in the absorption of chloride. The nature of the cellular activity responsible for this active absorption has been investigated further.

In the presence of a small amount of sulphate ion the chloride content of a loop of small intestine declines to as low as 5 per cent of the blood level of chloride in 90 minutes. When small quantities of other substances are added this process has been found to be altered. The following oxidation catalyst poisons, hydrogen sulphide, sodium arsenate, and sodium cyanide, all abolish or greatly diminish the phenomenon. The general protoplasmic poison, sodium fluoride, has a similar action. Sodium monooiodoacetate has either a very slight or no effect. On the other hand, several oxidation catalysts have been found to accelerate the flow of chloride into the blood against its diffusion gradient. Methylene blue and 2-4 dinitrophenol both have this effect.

The tentative conclusion may be drawn that the movement of the chloride ion is brought about by processes depending upon cell oxidations; whether the chloride itself enters into the oxidation process or not cannot be stated. It can be concluded justifiably, however, that in the absorption of chloride from the intestine the epithelium does not play a passive rôle.

Determination of the concentration of "heavy water" by means of the falling drop method. ELKIN VOGT (by invitation) and W. F. HAMILTON, Department of Physiology and Pharmacology, University of Georgia, School of Medicine, Augusta, Georgia.

The falling drop method of specific gravity determination of Barbour and Hamilton has been refined by means of accurate temperature control (to 0.001°C. for 30 min. intervals) and an improved pipette so that it is possible to determine the densities of 10 cu. mm. samples to within 2.5 parts per million. Thus a 10 cu. mm. drop of water can be "weighed" to within 0.000025 mgm.

Since "heavy water" can not be quantitated chemically this should prove a valuable aid to those who are working with this precious and physiologically significant material.

A preliminary report on the relation between falling time and small density differences will be presented.

The presence in normal human urine of a reticulocyte stimulating principle for the pigeon. G. E. WAKERLIN, H. D. BRUNER (by invitation) and J. M. KINSMAN (by invitation), University of Louisville School of Medicine.

1. A modification of the pigeon method for gauging the potency of anti-pernicious anemia preparations is outlined.
2. Normal human urine contains a substance which increases significantly the amount of reticular material in the red blood cells of the pigeon, as does also the erythropoietic principle present in liver.
3. Like the latter, this urinary substance is partially thermo-stable. Other evidence is presented pointing to at least a similarity, and possibly an identity, of the urinary and liver principles.
4. Work is in progress aimed at the obtainment of this urinary principle in suitable form for testing its erythro-genic properties in human pernicious anemia.

The effect of hepatectomy on the inorganic phosphate of the blood. E. T. WATERS (by invitation) and J. MARKOWITZ. Department of Physiology, University of Toronto.

Following the total removal of the liver, dogs received a continuous intravenous injection of dextrose in sufficient amount to maintain the concentration of the blood sugar at the pre-operative value. In these animals there was generally a progressive lowering of the blood inorganic phosphate over a period of several hours. Later the concentration of inorganic phosphate gradually increased until, just prior to the death of the animal, the concentration was approximately equal to or greater than the pre-operative value. Occasionally there was a transient increase in the inorganic phosphate concentration immediately succeeding the removal of the liver.

The action of acetyl-beta-methylcholine and epinephrine on the denervated coronary vessels. WILLIAM WEINSTEIN, KENNETH JOCHIM and ANNE BOHNING (introduced by Louis N. Katz). Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, Chicago.

We (Katz, Jochim and Bohning) have shown that changes in the coronary flow are produced by changes in the dynamic conditions under which the heart beats. In order to determine the effect of drugs on the coronary blood flow, uncomplicated by these extravascular effects, the ventricles of a denervated heart were thrown into permanent fibrillation and the coronary arteries perfused under constant pressure.

Acetyl-beta-methylcholine was found to cause an increase in coronary flow; in a few experiments this effect was absent. Acetylcholine, in the few experiments used, also caused an increased coronary flow. Epinephrine caused, in most experiments, an increase in coronary flow, in some, no effect, and in one experiment, a constriction when given after digitalis.

The vaso-dilator action of the acetylcholine derivatives can be readily explained as due to a "cholinergic action" on the parasympathetic endings; this action disappears after atropin. These results confirm our observations that the vagi are coronary vaso-dilators. The dilator action of epinephrine persists after atropin and ergotamine. It is therefore presumably not a typical "adrenergic" action on the sympathetic endings nor an action on the parasympathetic endings. This is being studied further.

The effect of choline on the oxygen consumption of normal and fatty livers of the rat. MARY SCOTT WELCH (by invitation), LAURENCE IRVING and C. H. BEST. Department of Physiology, University of Toronto.

Best and Huntsman have shown that choline, fed to rats having fatty livers, reduces the liver fat to a normal level. Likewise, a diet low in choline may cause large amounts of fat to accumulate in rat livers. The effect of choline on the oxygen consumption of normal and fatty livers has been investigated in an attempt to elucidate the mechanism by which choline reduces liver fat.

The oxygen consumption (Q_{O_2}) of normal rat livers in Ringer-Locke and glycerophosphate medium, pH 7.4, was found to be 8.54 ± 0.3 , while that of fatty livers was 5.88 ± 0.38 . The Q_{O_2} decreases regularly with increasing fat content. The slope of the curve for decrease in Q_{O_2} indicates that this decrease is not directly proportional to the fat content but that another factor besides the presence of fat is involved in the reduction of the Q_{O_2} .

Feeding choline to rats with fatty livers reduces the fat content of the liver to the normal value. These livers show a Q_{O_2} of 7.86 ± 0.76 , which is not significantly lower than the normal. Choline, added *in vitro*, increases the Q_{O_2} of normal livers to 12.98 ± 0.42 , and of fatty livers to 9.34 ± 0.45 . The accelerating effect of choline on the Q_{O_2} of fatty livers decreases with the increasing fat content at the same rate as does the oxygen consumption. The question of whether the choline is being oxidized by the tissue or is accelerating the oxidation of other substances in the liver has been investigated.

Factors influencing the sinking velocity of red cells. H. L. WHITE and BETTY MONAGHAN (by invitation). Department of Physiology, Washington University School of Medicine.

Increased aggregation of red cells causing increased sinking velocity occurs both in plasmas high in fibrinogen and in the presence of various agents, as gelatin. These might conceivably act 1, by forming an adsorption coat around the cell which might *a*, lower the zeta potential, or *b*, increase the cohesive force, or 2, by some process not dependent on adsorption. Adsorption is rendered unlikely but not disproved by Abramson's findings that red cells do not adsorb gelatin from 0.2 per cent solution and that horse cells show the same electrophoretic mobility in serum and in plasma. We have definitely disproved adsorption of fibrinogen at blood pH by finding 1, that the zeta of beef and of horse fibrinogen is about half that of the respective cells in fibrinogen solutions, and 2, that zeta of cells in M/10 phosphate buffer at pH 7.4 is not decreased but actually increased by addition of 2 per cent fibrinogen. However, fibrinogen is adsorbed at pH less than 5.6. Adsorption of gelatin even in 4 per cent solution is excluded since zeta of cells is not diminished but increased in gelatin, although zeta of gelatin at pH 7.4 is only $\frac{1}{3}$ to $\frac{1}{4}$ that of cells, the ratio varying with species. The increase in zeta is proportional to the gelatin concentration and is correlated with increase in sinking velocity and aggregation. Agents favoring aggregation apparently act by dehydrating the cell surfaces without themselves being adsorbed. This dehydration lowers stability by increasing probability of contact or cohesion or both in spite of a concomitant increase in zeta. Cells of different species differ in their susceptibility to dehydration, since horse cells aggregate more and sink faster in beef plasma than do beef cells in horse plasma and since horse cells sink faster than beef in a given gelatin solution. The sinking velocities and zetas of cells of various species in various gelatin solutions have been examined and some degree of correlation between sinking velocity and susceptibility to dehydration, as evidenced by increase in zeta, has been established.

The origin and significance of the neutral chloride present in the secretions of the stomach and duodenum. CHARLES M. WILHELMJ, LEO C. HENRICH (by invitation), IRWIN NEIGUS (by invitation) and FREDERICK C. HILL (by invitation). Departments of Physiology and Experimental Surgery, Creighton University School of Medicine, Omaha, Nebraska. (Read by title.)

The non-acid secretions of the stomach (pyloric secretion and fundic mucus) and the mixed duodenal secretions contain neutral chloride and are all mildly alkaline. Ninety-five experiments on whole stomach pouches, pyloric pouches, isolated duodenal pouches, secretions from the intact

duodenum and on dogs with gastroduodenostomy and gastrojejunostomy just below the ligament of Treitz in which approximately tenth normal hydrochloric acid solutions were either instilled into the pouches or mixed with their secretions have been analyzed. Under the conditions of these experiments it was found that approximately 30 per cent of the total neutral chloride represented neutralized hydrochloric acid while 70 per cent was a constituent of the secretions. This analysis shows that it is erroneous to consider all of the neutral chloride present in gastric contents as representing neutralized hydrochloric acid.

The influence of acid on the secretion of hydrochloric acid by the intact normal stomach. CHARLES M. WILHELMJ, F. T. O'BRIEN (by invitation) and FREDERICK C. HILL (by invitation). Departments of Physiology and Experimental Surgery, Creighton University School of Medicine, Omaha, Nebraska.

Wilhelmj, Neigus and Hill (This Journal, 1933) found that the presence of tenth normal hydrochloric acid in the intact stomach, or 0.173 normal hydrochloric acid in fundic pouches did not inhibit the secretion of hydrochloric acid *when histamine stimulation was used*. This was subsequently confirmed by Wilhelmj, Henrich and Hill (This Journal, 1934-1935) in a large series of experiments.

We have recently developed a test meal consisting of a two per cent aqueous solution of Liebig's extract containing phenol red. When this meal is given to normal dogs it causes a very marked secretion of hydrochloric acid and appears to be an ideal test meal. When the two per cent Liebig's extract is prepared in an acid solution instead of aqueous, it is found that the secretion of hydrochloric acid is inhibited, the degree of inhibition varying with the strength acid used. With a 0.06 normal acid no inhibition of secretion occurs but between 0.06 normal and 0.1 normal, progressive inhibition occurs which parallels the strength acid used. The degree of inhibition varies in different dogs but is either complete or very marked in all animals tried with 0.1 normal acid. It has been shown that this inhibitory effect is not due to destruction of the secretagogue in the Liebig's extract by the acid, nor is it due to the high chloride concentration of the solution. It has also been shown that the phenomenon is due to true inhibition and not to excessive neutralization of acid or to absorption of hydrochloric acid from the stomach. To date we have not determined whether this inhibition is solely an intragastric phenomenon or whether it involves a reflex mechanism from the stomach or duodenum.

Utilization of neutral fat by the Eck fistula dog. IRWIN C. WINTER (introduced by Lathan A. Crandall, Jr.). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Illinois.

Normal and Eck fistula dogs that had fasted three days were given 0.5 cc. linseed oil (iodine number 175) per kilo by stomach tube, and serum fatty acid and iodine number (I. N.) followed for five hours. The following days the procedure was repeated with the addition of 1 gram glucose per pound; this is known to depress the rise in blood fatty acids. In four of five normal dogs the serum fatty acids rose following oil alone, and in three instances this rise was accompanied by a rise in I. N. Fatty acids increased in only one of the four Eck fistula dogs, and in no case did these animals exhibit an increase in I. N.

When glucose was given with the fat no normal dog showed an increase in serum fatty acids, and the I. N. rose in only one and fell in one. In the Eck fistula animals two showed no change in fatty acids and two a distinct fall. There was a definite rise in I. N. in all four.

Following the administration of 4 cc. of linseed oil per kilo to five normal and three Eck fistula dogs there was uniformly a rise in serum fatty acid and I. N. in the normals. In two of the Ecks there was no change in fatty acid and only a slight rise in I. N., while the third (recently operated) behaved like the normals.

These results might lead one to question the ability of the Eck fistula animal to absorb fat, although the definite rise in I. N. in these animals when fed fat with glucose indicates either absorption of the linseed oil or selective removal of fatty acids of low I. N. If the linseed oil is absorbed the fatty acids with high I. N. must be selectively removed. Feeding glucose with linseed oil to normal animals evidently brings about more rapid removal from the blood stream of the oil that is fed, since both fatty acids and I. N. show less change than when oil alone is given.

Respiratory changes in the right and left ventricular pressure before and after the adult circulation is established, with a note on the diastolic and systolic pressures of the white mouse. R. A. WOODBURY (by invitation), W. F. HAMILTON and E. B. Woods (by invitation). Department of Physiology and Pharmacology and the Department of Obstetrics, University of Georgia, School of Medicine, Augusta, Georgia.

Simultaneous optical blood pressure tracings were taken from the right and left ventricles of unoperated rabbit fetuses. The positions of the cannulae were checked at autopsy. Before breathing and during the first exspirations the two ventricles had pressures nearly equal. Each early expansion of the lungs decreased the pulmonary resistance thus lowering the systolic pressure in the right ventricle by one half. In the left ventricle the systolic pressure remained relatively constant throughout the respiratory cycle. The excess left ventricular pressure proves that a functional valve exists in the ductus arteriosus, which was seen at autopsy.

The respiratory effect is totally different after the lungs are expanded. Respiration caused small changes in the pulmonary resistance and hence small changes in the right ventricular pressures. Expiration however resulted in a better filled left heart since both the diastolic and systolic pressures markedly increased. The foramen ovale is functionally closed a few hours after birth.

The adult white mouse has a heart rate of 720 and carotid pressures of 110/80 at inspiration and 125/90 at expiration.

Graded responses to graded stimuli in the turtle heart. GEORGE H. ZWIJKSTRA, JR. (introduced by T. E. Boyd). Loyola University School of Medicine, Chicago. (Demonstration.)

This is a demonstration of some of the phenomena observed in turtle hearts treated with excess KCl, as described in the accompanying abstract by Zwikstra and Boyd in the Proceedings.

Reversible loss of the all or none response in turtle hearts treated with excess of potassium. GEORGE H. ZWIJKSTRA, JR. (by invitation) and T. E. BOYD. Department of Physiology and Pharmacology, Loyola University School of Medicine, Chicago.

Turtle hearts, excised and placed in a modified Ringer solution containing from 0.120 to 0.240 per cent KCl, continue to respond to artificial stimuli for some time after the spontaneous beats have ceased. Before irritability is lost, perfect gradations of contraction are shown in response to graded stimuli. The condition develops earlier in the auricles than in the ventricles. The threshold is high, sometimes 5 volts or more, and maximum responses of the ventricle may require as much as 90 volts. Mixed effects are occasionally seen, in which a feeble all or none response, with low threshold, is obtained over the lower stimulus range, and superimposed on this a graded effect with stronger shocks. In the later stages, sustained contractions are obtained during passage of a constant current.

Hearts treated with excess potassium, but still beating at 20°, will on cooling stop at a temperature above 0°, varying with the concentration of potassium used. Auricles, below the temperature of cold standstill, show the phenomena described above. Spontaneous beats return on warming. Hearts from warm-stored turtles are more easily stopped by the combination of potassium and cold.

These effects of excess potassium are all reversible if the heart is transferred for a time to a standard Ringer solution (NaCl, 0.70, CaCl₂, 0.025, KCl 0.40 per cent). They are not reversed nor prevented by a proportionate increase in the Ca content of the modified Ringer.

The potassium content of the turtle ventricle was found to average 310 mgm. per hundred grams of tissue (wet weight) as determined by a modification of the Breh and Gaebler quantitative method. In studies on the potassium balance, solutions which bring on the graded type of response are usually found to have lost potassium to the ventricle.

A new electrocardiograph. F. L. DUNN and J. P. TOLLMAN (introduced by A. R. McIntyre). The Departments of Physiology and Pharmacology and Clinical Research, University of Nebraska Medical College, Omaha, Nebraska. (Demonstration.)

The recent development of vacuum tubes of high sensitivity, greater mechanical strength and insensitivity to extraneous electrical disturbances permits the construction of amplifiers of greater sensitivity and fidelity than hitherto possible. In its preliminary form the instrument is capable of producing electrocardiographs of high fidelity and greater amplitude than those commonly available. The circuit does not differ materially from conventional audio-frequency practice; however, the degree of amplification obtained permits the use of more rugged oscillographs and the recording of finer detail of wave form, advantages of possible significance in the recognition of early myocardial damage.

THE RELATION OF THE PARATHYROID HORMONE TO THE STATE OF CALCIUM IN THE BLOOD¹

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The literature concerning the relation of the parathyroid glands to the behavior of calcium in the organism has recently been reviewed by Thomson and Collip (1932), making an extensive review unnecessary here. Experimental evidence bearing directly upon the work reported in this paper will be considered as a part of the discussion.

There has been no unanimity of opinion with reference to the effects of removal of the parathyroid glands, or of the administration of the parathyroid hormone, upon the state of calcium in the serum, owing chiefly to the fact that the problem has necessarily been attacked by indirect methods. In the absence of a satisfactory understanding of the state of calcium in the serum under normal conditions, interpretation of observations under abnormal conditions has been doubly difficult.

A new approach to the problem has been made possible by the introduction, by two of the authors (McLean and Hastings, 1934), of a method for the direct estimation of calcium ion concentrations, applicable to the fluids of the body, and by a description of the conditions affecting the ionization of calcium in normal serum (McLean and Hastings, 1935).

EXPERIMENTAL. The object of the experiments to be reported was to make observations upon the state of calcium in the serum in conditions approximating, as closely as possible, hypo- and hyperfunction of the parathyroid glands. Hypofunction was simulated by thyro-parathyroidectomy, hyperfunction by subcutaneous administration of the parathyroid hormone (parathyroid extract, Lilly).

For the most part the experiments were performed on cats, blood for the necessary observations being obtained by heart puncture, without anesthesia.

A few observations upon chronic latent tetany in the dog are also included. The results of a limited number of observations upon human

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serum made in coöperation with Dr. W. O. Thompson, and to be reported by him, make it appear that the conclusions of this paper may safely be applied to human material.

The observations upon serum consisted of direct observation of Ca^{++} concentrations by the biological method previously described (McLean and Hastings, 1934), and of analysis for total calcium and total protein, from which Ca^{++} concentrations were calculated nomographically from the mass-law equation

$$(1) \quad \frac{[\text{Ca}^{++}] \times [\text{Prot}^-]}{[\text{CaProt}]} = K$$

previously shown (McLean and Hastings, 1935) to represent a satisfactory first approximation of the conditions present in normal human serum. In logarithmic form this equation is written

$$(2) \quad p\text{Ca}^{++} + p\text{Prot}^- - p\text{CaProt} = pK_{\text{CaProt}}$$

in which p is the negative logarithm.

From eight observations on normal cats, previous to the initiation of any experimental procedures a mean value of $2.25 \pm$ standard deviation 0.05 was obtained for pK_{CaProt} in equation 2, which is sufficiently close to the value of $2.22 \pm$ standard deviation 0.07 obtained from human material to warrant the conclusion that the conditions in normal human serum and in serum from the normal cat are, within the limits of accuracy of the methods, identical. Consequently the value of 2.22 for pK_{CaProt} has been used throughout this paper in calculating Ca^{++} concentrations from the results of analysis for total protein and total calcium.

The mean values for Ca^{++} concentrations in the serum of the normal cat, as obtained by the two methods, showed reasonably satisfactory agreement. By giving equal weight to observed and calculated values a mean value of $1.15 \pm$ standard deviation 0.07 mM per kilo H_2O was obtained from this small series of observations, a value slightly lower than that of 1.25 ± 0.09 similarly obtained in the case of normal adult man (McLean and Hastings, 1935).

Total calcium was determined by the method of Kramer and Tisdall (1921) as modified by Clark and Collip (1925). Total nitrogen was determined by the Kjeldahl method. Total protein was calculated from total less non-protein nitrogen, using a factor of 6.3 to convert grams of nitrogen to grams of protein. An arbitrary correction of 30 mgm. per 100 cc., for non-protein nitrogen, was used. That this assumed correction does not introduce an appreciable error, particularly in view of the rise in non-protein nitrogen reported after administration of parathyroid extract to dogs, was found by actual analysis for non-protein nitrogen in the serum of two of the cats studied.

The protocol of a representative complete experiment, including all observations upon one cat, is reproduced in table 1.

Thyro-parathyroidectomy. Of seven cats operated upon, six responded by lowering of the total serum calcium to 1.4 mM per liter or less, and three developed tetany. The seventh cat exhibited no tetany, and no decrease in the total calcium of the serum was observed. Twenty observations made upon cats with total calcium in the serum below 1.75 mM per liter

TABLE 1

Calculated and observed Ca^{++} concentrations, serum, cat D, thyro-parathyroidectomy and administration of parathyroid extract

Ca^{++} calculated from $\text{pK}_{\text{CaProt}} 2.22$

DATE	DILU-TION	TOTAL PRO-TEIN	TOTAL CALCIUM	Ca^{++}			$\text{pK}_{\text{CaProt}}$	REMARKS
				Calcu-lated	Ob-served	Differ-ence		
1934	per cent serum	gm. per 100 cc.	mM per kgm. H_2O					
9/24	100	6.9	2.69	1.20	1.20		2.22	Normal control
	50	3.45	1.31	0.89	0.89		2.22	Thyro-parathyroidectomy 9/25
9/27	100	7.6	1.63	0.66	0.65	-0.01	2.23	
9/29	100	7.4	1.31	0.52	0.55	+0.03	2.19	9/29, no symptoms;
10/1	100	7.6	1.52	0.61	0.60	-0.01	2.23	9/30, spastic and some
10/3	100	7.8	1.70	0.68	0.65	-0.03	2.25	tetany; 10/1, in-
10/5	100	7.4	2.04	0.86	0.90	+0.04	2.17	creased tetany; 10/2,
	50	3.7	0.99	0.59	0.60	+0.01	2.18	no symptoms
10/8	100	7.3	2.32	0.98	1.00	+0.02	2.20	100 units parathyroid
	50	3.65	1.12	0.66	0.70	+0.04	2.16	extract daily 10/1
10/10	100	7.0	2.86	1.28	1.20	-0.08	2.27	10/12, total 1200 units
	50	3.5	1.39	0.85	0.85		2.22	
	25	1.75	0.68	0.52	0.50	-0.02	2.26	
10/13	100	7.2	3.83	1.73				Depressed and flaccid
	50	3.6	1.86	1.14	1.25	+0.11	2.09	
	25	1.8	0.92	0.70	0.80	+0.10	1.86	
10/15	100	6.8	2.62	1.18	1.20	+0.02	2.20	
	50	3.4	1.28	0.79	0.90	+0.11	2.03	
10/20	100	7.3	1.25	0.51	0.50	-0.01	2.23	No symptoms
10/23	100	6.5	1.04	0.45	0.42	-0.03	2.28	Very spastic, not much tetany. Died 10/24

showed satisfactory agreement between values for Ca^{++} as obtained by direct observation and by calculation from total calcium and total protein, the average deviation being ± 0.05 mM per kilo H_2O . The mean value for $\text{pK}_{\text{CaProt}}$, as calculated from direct observation of Ca^{++} concentrations in this series, was $2.24 \pm$ standard deviation 0.08.

Thomson and Collip (1932) have pointed out that "there is no definite level of the [total] serum calcium at which tetany may be expected."

Our observations confirm the absence of any definite correlation between the total calcium level and the occurrence of tetany, but also indicate a similar lack of correlation between the Ca^{++} concentration and the appearance or severity of tetany, except for the fact that tetany was not observed at Ca^{++} concentrations above 0.65 mM per kilo H_2O . In general, as illustrated by table 1, the onset of tetany did not coincide with the first observation of the Ca^{++} level at or above which tetany subsequently appeared. Moreover, the prompt relief from symptoms following administration of parathyroid hormone does not appear to have been necessarily accompanied by any immediate and striking increase in the Ca^{++} concentration in the serum. These observations, reinforced by the observations in chronic latent tetany in the dog, reported in this paper, indicate that the Ca^{++} concentration must be considered as being only one of the variables concerned in the occurrence of tetany following parathyroidectomy.

Administration of parathyroid extract. Observations were made following the subcutaneous administration of parathyroid extract to normal cats and to cats previously thyro-parathyroidectomized. In general a uniform daily dosage of 100 new standard units, corresponding to 20 old units, was employed. All of 10 cats so treated responded with a rise in the calcium level to 3.22 mM liter or higher. Five cats exhibited symptoms, and two died with the typical symptoms of hyperparathyroidism. The time elapsing between beginning the injections and attainment of the characteristic high level varied from 3 to 10 days, and appeared to bear no relation to the previous state of the animals, the same variations being noted in previously normal animals, and in animals in tetany following thyro-parathyroidectomy. That these variations were not due to variability in the potency of the extract used was shown by the fact that they occurred among animals receiving the same preparation. This variability is being further investigated.

It was usually not found possible to make direct comparisons between observed and calculated Ca^{++} concentrations in undiluted hyperealeemic serum. That the mass-law equation 1 is valid for human serum diluted with a saline solution has previously been shown (McLean and Hastings, 1935). In the observations upon diluted serum from normal cats (50 per cent and 25 per cent serum), included in figure 1, the mass-law equation was found to predict the observed Ca^{++} concentration within the limits of accuracy of the methods.

Eleven observations were made upon cats with total calcium in the serum above 3.2 mM per liter following administration of parathyroid extract. In only one case (calculated Ca^{++} , 1.83 mM per liter; observed Ca^{++} , 1.75 mM per liter) was it possible to make a satisfactory direct observation upon undiluted serum. The remaining observations were made

upon serum diluted to 50 per cent and 25 per cent. In the 11 observations on serum diluted to 50 per cent the average deviation between observed and calculated Ca^{++} concentrations was ± 0.08 , and the same series of observations gave a mean value of $2.20 \pm$ standard deviation 0.08 for pK_{CaProt} in equation 2.

Correlation of calculated and observed Ca^{++} concentrations. Figure 1 illustrates 96 observations, upon undiluted and diluted serum from the cat, in which calculated and observed Ca^{++} concentrations could be compared. There is shown a high degree of correlation between observed values for Ca^{++} and values calculated from equation 2, using a value of 2.22 for

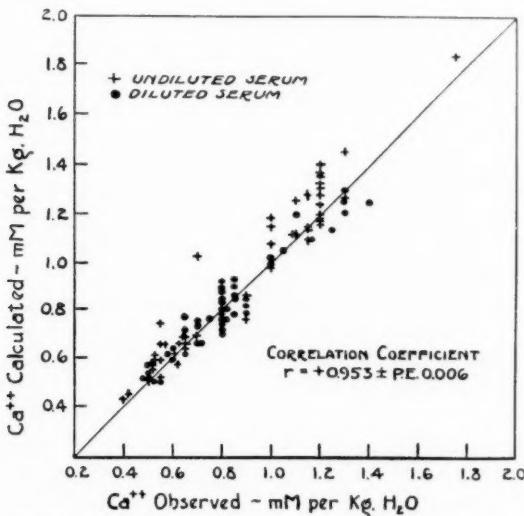


Fig. 1. Observed and calculated Ca^{++} concentrations in cat's serum, whole and diluted.

pK_{CaProt} . The data upon which figure 1 is based show an average deviation of ± 0.06 mM per kilo H_2O between calculated and observed Ca^{++} concentrations, and a mean value of $2.23 \pm$ standard deviation 0.10 for pK_{CaProt} , which is in close agreement with the results from human serum (McLean and Hastings, 1935). With few exceptions, the correspondence between observed and calculated values for Ca^{++} is as close as may be expected, in individual cases as well as statistically.

From the data as summarized in figure 1, it may be concluded that over the entire range from minimum hypocalcemia, following thyro-parathyroidectomy, to maximum hypercalcemia, following the administration of parathyroid extract, the changes in the state of the calcium found in the

serum are simply the quantitative changes predicted by the mass law equation, and that there is in these experiments, in the absence of any consistent deviation between observed and calculated Ca^{++} concentrations, no evidence of any qualitative change in its state.

Latent tetany in the dog. As first shown by Luckhardt and Goldberg (1923) a state of chronic latent tetany, without treatment and without symptoms, may be produced in the dog, provided that, by suitable treatment, death in the acute state following parathyroidectomy is prevented. Dragstedt and Sudan (1926) reported serum calcium figures, in such animals, as low as 4 mgm. per 100 cc. three months after thyro-parathyroidectomy, and Dragstedt (1927) suggested that "after a varying period of four to six weeks the nervous system becomes so adjusted to the altered blood that tetany no longer results when treatment is stopped." Since there has remained the possibility that a chemical adjustment, resulting in a change in the calcium-protein relationship, occurs in these animals (Klinke, 1931, p. 253) it has seemed desirable to make direct observations of Ca^{++} concentrations in the plasma or serum in chronic latent tetany.

As previously reported (McLean and Hastings, 1934) serum from the dog is toxic to the frog's heart. Observations upon the Ca^{++} concentration of such serum are attended with some difficulty, but may be made at the cost of frequent renewal of preparations. During the course of our studies it was discovered that plasma from the dog, with the use of sodium polyanethol-sulphonate² as an anticoagulant, is non-toxic to the frog's heart, and that use of this substance does not affect the ionization of calcium. Table 2 includes observations upon both serum and plasma, and indicates that the mass law equation 1 holds in the case of chronic latent tetany, and that consequently the organism in this condition is adjusted to Ca^{++} concentrations which in the acute stage of hypoparathyroidism would ordinarily be associated with severe tetany. It is worthy of note that total calcium concentrations of 3.8, 3.6, 3.2 and 3.7 mgm. per 100 cc. of plasma were observed, in two of the dogs studied, without treatment and without tetany. These observations were made shortly after the termination of calcium therapy, and correspond with the low levels of 3.5 mgm. for serum and 3.2 mgm. for plasma reported by Morgulis and Perley (1930) under similar conditions.

DISCUSSION. Trendelenburg and Goebel (1921) demonstrated that serum from thyro-parathyroidectomized cats diminished the amplitude of contraction of the isolated frog's heart, as compared with serum from normal cats, and that addition of appropriate amounts of calcium to the serum

² "Liquoid-Roche," obtained from Hoffmann-LaRoche, Inc., Nutley, N. J. If this preparation is used it is also possible to introduce whole blood into the frog heart preparation and thus to obtain observations upon the Ca^{++} concentration of the plasma.

increased the amplitude to that induced by normal serum. They concluded that the reduction in amplitude of contraction of the frog's heart was due to a decrease in the total calcium of the serum, with a corresponding decrease in the ionized fraction, but were unable to rule out the possibility of the formation of an un-ionized calcium compound, presumably by combination with an organic acid. The experiments here reported, combined with the previous evidence advanced by two of the authors (McLean and Hastings, 1935) to the effect that the sensitivity of the frog's heart to changes in the calcium content of its nutrient fluid is specific for ionized calcium, add quantitative strength to the first conclusion of Trendelenburg and Goebel, and in addition exclude the possibility of the

TABLE 2
Observations on serum and plasma in chronic latent tetany in the dog

DOG	DATE	MATERIAL	TOTAL PROTEIN	TOTAL CALCIUM	Ca ⁺⁺			REMARKS
					Calculated	Observed	Difference	
			gm. per 100 cc.	mM per kgm. H ₂ O				
S*	7/16/34	Serum	5.9	1.27	0.59	0.60	+0.01	No tetany
	9/26/34	Serum	6.7	1.68	0.74	0.70	-0.04	No tetany
	10/20/34	Serum	7.1	1.40	0.58	0.75	+0.17	No tetany
	12/5/34	Plasma	7.5	1.46	0.59	0.65	+0.06	No tetany
E†	12/11/34	Plasma	6.5	1.48	0.65	0.60	-0.05	Acute tetany
	2/11/35	Plasma	6.4	1.01	0.43	0.50	+0.07	No tetany
	2/13/35	Plasma	5.9	0.96	0.44	0.40	-0.04	No tetany
	2/25/35	Plasma	5.8	1.15	0.54	0.35	-0.19	No tetany
L‡	2/6/35	Plasma	5.9			0.35		No tetany
	2/11/35	Plasma	6.0	0.84	0.37	0.42	+0.05	No tetany
	2/13/35	Plasma	5.4	0.98	0.45	0.35	-0.10	No tetany

* Thyro-parathyroidectomy 1/24/34, calcium therapy for 2 months.

† Thyro-parathyroidectomy 12/9/34, no calcium after 2/6/35.

‡ Thyro-parathyroidectomy 12/16/34, no calcium after 1/31/35.

accumulation of any abnormal calcium compounds in the blood, in quantitatively significant amounts, either following thyro-parathyroidectomy or following the administration of parathyroid extract.

From our previous studies (McLean and Hastings, 1935) it would appear that diffusible calcium, as determined by compensation dialysis and ultrafiltration methods, is at least a rough measure of Ca⁺⁺ concentration. There have been numerous studies of the diffusible calcium in clinical hypoparathyroidism, and following parathyroidectomy or the administration of the parathyroid hormone (see Thomson and Collip, 1932, for literature). The general results of these studies are in entire agreement with those here reported, in that both diffusible and non-diffusible calcium are found to be diminished in hypofunction and increased in hyperfunction

of the parathyroid glands. As to the ratio between diffusible and total calcium, the results are not in complete agreement, but by most investigators no consistent change in this ratio, in either hypo- or hyperparathyroidism, could be found. While the variability in the results as reported cannot be used as positive evidence of approximate constancy of the ratio, failure to demonstrate consistent changes in this ratio is in accord with the findings and conclusions in this paper.

Collip (1926), after having concluded that the function of the parathyroid glands is to maintain a constant level of calcium in the plasma, wrote, "It is the opinion of this writer that the absolute concentration of calcium ions is the most important factor in calcium metabolism, and that this factor is normally regulated either directly or indirectly by the parathyroid glands." The findings here reported appear to confirm this opinion. Since, in the observations here reported, total protein fluctuates but slightly, it is impossible from these experiments alone to distinguish between regulation of total calcium and of Ca^{++} concentrations. From studies previously reported (McLean and Hastings, 1935) it appears that, in the absence of disorders of the parathyroid glands, variations in total protein are accompanied by changes in total calcium, the result being approximate constancy of Ca^{++} concentrations, and that this may be considered to be the normal physiological response. It may therefore be concluded that the regulating mechanism is directed toward the maintenance of the Ca^{++} concentration at a physiological level. It should be made clear, however, that the Ca^{++} concentration in the plasma at any time is determined by the total calcium and total protein concentrations present at the moment, and that to this extent the Ca^{++} concentration in the plasma is solely a physical-chemical function of the conditions present in the blood. The regulatory mechanism, then, functions so as to bring about such conditions in the blood as will yield favorable concentrations of Ca^{++} . That the parathyroid hormone plays a decisive part in this regulatory process would seem to be fully established.

It now appears, from the studies reported here and previously, that the Ca^{++} concentration in the plasma can be considered to be only one of the variables concerned in the symptoms of hypoparathyroidism, and presumably also of hyperparathyroidism. The hypothesis that other factors, such as acid and alkali, known to influence the incidence or course of tetany, act solely by influencing the ionization of calcium in the plasma, appears to be no longer tenable. With definition of the Ca^{++} concentration now possible, study of the rôle of other factors should be facilitated.

SUMMARY

1. The mean Ca^{++} concentration in the serum of 8 normal cats was found to be $1.15 \pm$ standard deviation 0.07 mM per kilo H_2O . The ex-

tremes of the normal range may be put tentatively at 1.05 and 1.25 mM per kilo H_2O .

2. Ionization of calcium in the serum of the normal cat follows, as a first approximation, the mass-law relationship previously described for normal human serum, without change in the value for the constant.

3. This relationship also holds over the entire range from the hypocalcemia following thyro-parathyroidectomy, including that associated with tetany, to the maximum hypercalcemia following the administration of parathyroid extract. Consequently the changes in the state of the calcium found in the serum under these conditions are simply the quantitative changes predicted by the mass-law relationship, there being no evidence of a qualitative change in its state.

4. A regulatory mechanism, in which the parathyroid hormone plays a decisive part, functions so as to maintain the Ca^{++} concentration of the plasma at a physiological level. Removal of the parathyroid glands leads to a reduction in Ca^{++} concentration, administration of parathyroid hormone to an increase.

5. Acute post-operative parathyroid tetany, in the cat, was observed only when the Ca^{++} concentration in the serum was found to be 0.65 mM per kilo H_2O or below, and then only irregularly. Neither its occurrence nor its severity could be further correlated with the Ca^{++} concentration in the serum.

6. The symptoms of hyperparathyroidism may appear, in the cat, when the Ca^{++} concentration in the serum rises above 1.7 mM per kilo H_2O , and death may occur at Ca^{++} concentrations between 1.7 and 2.0 mM per kilo H_2O .

7. Chronic latent tetany, in the dog, is accompanied by a chronic lowering of the Ca^{++} concentration in the plasma.

8. The use of sodium polyanetholsulphonate as an anticoagulant does not affect the ionization of calcium, and plasma so obtained from dog's blood is not toxic to the frog's heart.

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TOTAL PLASMA PROTEIN IN NORMAL AND FASTING RATS¹

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The study of the changes in plasma protein concentration of rats cannot measure the variations in total blood protein, since plasma volume is unknown in such determinations. The dye dilution method for plasma volume estimation is less applicable in rats than in larger animals where the removal of enough blood for colorimetric determinations does not reduce the total blood volume perceptibly. The complete washing-out technique, similar to that employed by Weleker (1) in 1858, and subsequently followed by other investigators, would appear to be satisfactory if all the red cells could be removed by perfusion; for then, if the ratio of cells to plasma in an initial sample were known, the total plasma volume could be calculated.

In practice this is done by bleeding a rat of a considerable portion of its blood volume, and then washing out as nearly as possible all the remaining red cells as a separate second specimen. The ratio of cell volume to plasma volume of the first sample and the red cell volume of the second sample are obtained by hematocrit estimations, and from these data the total volume is calculated.

This paper presents such a method, the normal plasma and plasma protein values obtained by its use, and the effect of fasting on these values.

1. *A method of total blood protein estimation by means of blood volume determinations: Blood volume method.* The rat is anesthetized with ether and weighed. The jugular veins are dissected free, and the entire head of the animal is greased with vaseline. The rat is placed belly up on a low box with its head hanging over the edge, both jugulars are ligated, a few drops of saturated sodium citrate solution from a tuberculin Luer are dropped on one vein, the vein is nicked distal to the ligature, and blood collected in a small beaker just moistened with the citrate. Three to 6 cc. of blood are collected while citrate is slowly dropped from the syringe onto the vein. The total volume of citrate used, usually about 0.1 cc., is recorded. This is specimen I.

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When the desired amount of blood has been collected for specimen I the beaker is removed and a larger beaker or flat dish containing a few cubic centimeters of isotonic (1.6 per cent) sodium citrate is quickly placed under the head and the bleeding vein washed frequently throughout the remainder of the procedure with an unmeasured amount of isotonic citrate. A needle (gauge 22) connected by a rubber tubing to a burette of Ringer's solution is at once tied into the other jugular vein, directed toward the heart, and a slow flow started. After perfusion of 50 to 75 cc. the fluid coming from the jugular (or jugulars, if both are cut) is practically clear. This completes specimen II and removes practically all the grossly visible red cells from the rat except from the spleen.

Continued perfusion gives a nearly clear fluid which contains protein presumably washed in from the tissues, and constitutes specimen III.

The total volume of specimen II is recorded; then all of specimen I and about 12 cc. each of specimens II and III are transferred to centrifuge tubes and spun at 2,500 r.p.m. for 30 minutes. Fifty cubic centimeter samples of specimen I and II are also centrifuged in large tubes to give adequate plasma for protein analysis.

After centrifugalization the plasmas are pipetted off and the red cells shaken up with normal salt solution and recentrifuged in order that the cell volume may be measured under isotonic conditions to make comparison possible. This measurement will be called the corrected cell volume.

The readings and calculations are recorded as in the following protocol:

Rat 2. Male, 202 grams; 391 sq. cm. body surface

<i>Specimen I</i>	<i>Specimen II</i>	<i>Specimen III</i>
Total volume 4.05 cc.	Total volume II. 60.0 cc.	Total volume 20.0 cc.
Cell volume <u>1.75 cc.</u>	Vol. of centrifri- fuged portion of II 13.50 cc.	
"Plasma I" (plas- ma plus citrate). 2.30 cc.	Cell volume <u>0.48 cc.</u>	
Citrate volume 0.13 cc.	Plasma volume of centrifuged portion of II. 13.02 cc.	
Plasma volume I. 2.17 cc.	Corrected cell volume of cen- trifuged por- tion of II. 0.48 cc.	
Corrected cell volume I. 1.90 cc.		

$$\text{Plasma vol. II} = \frac{\text{Plasma vol. I} \times \text{corrected cell vol. of centrif. portion of II}}{\text{Corrected cell vol. I} \times \text{vol. of centrif. portion of II}} \times$$

$$\text{Total volume II} = \frac{2.17 \times .48 \times 60}{1.9 \times 13.5} = 2.44 \text{ cc.}$$

Then, Plasma volume I = 2.17 cc.

Plasma volume II = 2.44 cc.

Total plasma volume = 4.61 cc.

RESULTS. In 25 rats the average total plasma protein as determined by this method was 0.0983 gram per 100 sq.cm. body surface.

Using this method, the average plasma volume of 25 normal rats was 1.4 cc. per 100 sq.cm. of body surface. Figure 1 shows the correlation between surface area and plasma volume in 51 male rats, including both normal and starved animals. The individual values for plasma volume per 100 sq.cm. of body surface shown in table 1 indicate that the relationship is not linear; thus larger rats have an increase in plasma volume more than directly proportional to body surface.

Total protein method. Total plasma protein concentration is measured by the gravimetric method of Barnett, Jones and Cohn (2), with the alter-

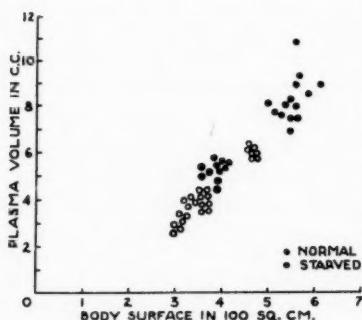


Fig. 1

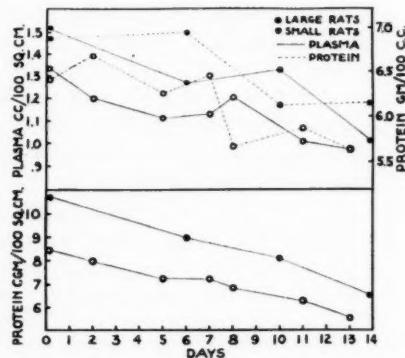


Fig. 2

Fig. 1. The relationship of plasma volume to body surface for normal and starved rats.

Fig. 2. Above: Plasma volume and plasma protein concentration changes in starved rats.

Below: Total plasma protein changes in starved rats.

ation of washing the precipitated protein with the acetate buffer rather than with distilled water. To lessen errors of estimation in the dilute solutions, larger amounts of specimens II and III are used than of specimen I. The usual amounts are: specimen I, 1.0 cc. plus 5.0 cc. of the acetate buffer; specimens II and III, 20.0 cc. plus 10.0 cc. buffer plus 0.1 gram sodium chloride. Specimen II is a dilution of about 1:20 and specimen III about 1:200 of the original blood. In these dilute determinations a small amount of protein is rather consistently lost. As determined from known dilutions of normal blood, the value for specimen II is multiplied by 1.05, and the value for specimen III by 1.10 to correct for this loss.

In calculating total protein content, specimen I must be corrected for dilution by citrate, and specimen II for the volume of red cells as shown in the protocol below. Specimen III needs no such correction.

The calculated total blood protein as represented by the product of the total plasma volume and the protein concentration in specimen I is always exceeded by the actual sum of the total protein determined by adding together that found in specimens I and II, presumably due to the addition of protein washed out of the tissues by the perfusion and present in specimen II.

The protein readings and calculations are recorded as in this continuation of the previously cited protocol.

Specimen I:

$$\begin{aligned} \text{Per cent concentration of protein} &= \text{weight of protein in 1 cc. plasma} \times \text{citrate} \\ &\quad \text{correction} \\ &= 0.0652 \times \frac{2.30}{2.17} = 0.0690 \text{ gram per cc.} \end{aligned}$$

$$\begin{aligned} \text{Total plasma protein} &= \text{per cent conc. of protein} \times \text{plasma volume} \\ &= 0.0690 \times 4.61 = 0.318 \text{ gram} \end{aligned}$$

$$\begin{aligned} \text{Total plasma I protein} &= \text{per cent conc. protein} \times \text{plasma I volume} \\ &= 0.0690 \times 2.17 = 0.150 \text{ gram} \end{aligned}$$

Specimen II:

$$\begin{aligned} \text{Total plasma II protein} &= \frac{\text{wt. protein in 20 cc. of specimen II} \times \text{total vol. II}}{\text{vol. of centrifuged portion of II} \times 20 \text{ cc.}} \\ &\quad \times \text{plasma vol. of centrif. portion of II} \times 1.05 \\ &= \frac{0.0767 \times 13.02 \times 60 \times 1.05}{13.50 \times 20} = 0.233 \text{ gram} \end{aligned}$$

$$\begin{aligned} \text{Protein from tissues} &= \text{total plasma I protein} + \text{total plasma II protein} \\ &\quad - \text{calculated total plasma protein} \\ &= 0.150 + 0.233 - 0.318 = 0.065 \text{ gram} \end{aligned}$$

Specimen III:

$$\begin{aligned} \text{Per cent concentration of protein} &= \frac{\text{wt. protein in 20 cc. spec. III} \times 1.10}{20} \\ &= \frac{0.0114 \times 1.10}{20} = 0.00063 \text{ gram per cubic} \\ &\quad \text{centimeter} \end{aligned}$$

The amounts of protein presumably washed in from the tissues in different rats are only roughly comparable, since the washing was not always quantitated either in time or amount. The average value was 0.0208 gram per 100 sq.cm. body surface.

In specimen III there was an average protein concentration of 0.67 gram per cent, the value varying widely with the rapidity and duration of the perfusion.

Table 1 records the individual values for plasma volume and protein from two groups of rats, one of large and one of small animals.

Albumin and globulin method. Albumin values are obtained by the usual method of half saturation with ammonium sulfate; globulin by subtracting these from total protein values. By this method correct albumin and globulin values are obtained in undiluted blood, but control determinations show the results for albumin obtained in the dilute specimens II and III to be too high. Specimen II values must be multiplied by a

TABLE 1

	SURFACE AREA	PLASMA VOLUME	PLASMA VOLUME PER 100 SQ. CM.	PER CENT CONCENTRATION PROTEIN IN PLASMA	TOTAL PLASMA PROTEIN PER 100 SQ. CM.	TOTAL* PROTEIN WASHED IN FROM TISSUES PER 100 SQ. CM.	PER CENT CONCENTRATION PROTEIN IN SPECIMEN III
Large rats†							
	sq. cm.	cc.	cc.	gm. per 100 cc.	gm.	gm.	gm. per 100 cc.
Maximum.....	611	10.82	1.930	7.09	0.1250	0.0432	
Minimum.....	500	7.53	1.362	6.32	0.0884	0.0237	
Mean.....	552	8.23	1.508	6.88	0.1079	0.0262	
Small rats‡							
Maximum.....	404	5.46	1.393	6.90	0.0913	0.0221	0.104
Minimum.....	373	4.61	1.180	6.18	0.0768	0.0098	0.047
Mean.....	386	5.04	1.307	6.30	0.0825	0.0167	0.071

* For 8 rats.

† For 14 rats.

‡ For 16 rats.

correction factor of 0.75, and specimen II values by 0.55. All albumin and globulin values in this paper have been so corrected. Since the degree of dilution and hence the factor to be applied vary somewhat, these diluted estimations are only approximate.

RESULTS. Table 2 shows the albumin and globulin values for the rats whose total protein values are shown in table 1.

The average albumin-globulin ratio for the undiluted blood of 16 normal rats was 0.91:1; for specimen II, 1.03:1. Estimations on specimen III were too few to be conclusive and varied greatly, with an average of 0.43:1.

2. *The effect of fasting on circulating proteins:* A series of large and

another of small rats were fasted for periods up to two weeks; water was not restricted. Plasma volume and protein studies were made at various intervals. Since it was possible that the rats received some nourishment by eating their own stools, one group was fasted two days in a refection experiment over a large-mesh wire screen. Their total protein was 0.0755 gram per 100 cc. while that of rats starved with access to their stools was 0.0805 gram per 100 cc.

RESULTS. The total plasma protein fell in each group as a linear function of time as shown in the lower half of figure 2; the upper half shows the compensating fluctuations in total plasma volume and protein concentration which allow the composite curve of total plasma protein to be linear. Individual results are shown in table 3.

TABLE 2

	SPECIMEN I			SPECIMEN II			SPECIMEN III*		
	Albu-min	Globu-lin	Ratio	Albu-min	Globu-lin	Ratio	Albu-min	Globu-lin	Ratio
Large rats†									
	gm. per cent	gm. per cent		gm. per cent	gm. per cent		gm. per cent	gm. per cent	
Mean.....	3.29	3.63	0.90:1	0.145	0.265	0.55:1			
Small rats‡									
Maximum.....	3.18	3.62	1.07:1	0.198	0.214	1.78:1	0.03	0.06	0.75:1
Minimum.....	2.62	2.90	0.73:1	0.130	0.111	0.68:1	0.02	0.04	0.30:1
Mean.....	2.98	3.35	0.91:1	0.162	0.167	1.03:1	0.02	0.05	0.43:1

* For 7 rats.

† For 2 rats; serum pooled.

‡ For 16 rats.

The albumin-globulin ratio of the whole blood fell rather abruptly as shown in table 4 and remained on a reduced, but roughly level plateau throughout the experiment. In specimen II the same fall is seen but with less regularity. A few estimations on specimen III show a rather marked fall in albumin with the progress of fasting.

DISCUSSION. The significance of these experiments falls into two categories; the first concerns the total plasma protein, and the second the protein washed into the blood stream from the tissues during the perfusion.

1. *Total plasma protein and its fractions:* With a method of blood volume estimation as a tool, the total plasma protein may be followed through an experiment without the aberration inherent when only protein concentrations are known. Thus dehydration, or changes in osmotic pressure which may mask concentrations are of less disturbance. Also among normal rats

a lack of perfect linear relationship between size of body surface and plasma volume is seen. Small rats have less plasma per unit surface area than large rats. In fasting this discrepancy is exaggerated. Thus compensating concentration changes may be misleading.

How these various factors may influence concentration and volume is shown in the fasting experiments described. It is seen that protein concentrations remain high for about a week and then fall to a lower level at which they remain until death, while volume changes occur more

TABLE 3
Plasma volume and plasma and tissue space protein changes in starvation

NUMBER OF RATS	SURFACE AREA	PLASMA VOLUME	PLASMA VOLUME PER 100 SQ. CM.	PER CENT CONCENTRATION PROTEIN IN PLASMA	TOTAL PLASMA PROTEIN PER 100 SQ. CM.	TOTAL PROTEIN WASHED FROM TISSUES PER 100 SQ. CM.	PER CENT CONCENTRATION PROTEIN SPECIMEN III
Large rats							
		sq. cm.	cc.	cc.	gm. per 100 cc.	gm.	gm. per cent
0	14	552	8.23	1.508	6.88	0.1079	0.0262
6	4	465	5.97	1.283	6.95	0.0893	0.0206
10	3	464	6.15	1.325	6.12	0.0811	0.0120
14	4	364	3.86	1.060	6.14	0.0652	0.0151
Small rats							
0	16	386	5.04	1.307	6.30	0.0825	0.0167
2	5	370	4.44	1.200	6.70	0.0805	0.0159
2*	5	387	5.01	1.295	5.83	0.0755	0.0250
5	4	347	3.99	1.150	6.26	0.0721	0.0179
7	5	323	3.64	1.126	6.48	0.0730	0.0192
8	4	360	4.33	1.205	5.66	0.0684	0.0114
11	3	317	3.36	1.060	5.86	0.0621	0.0123
13	4	310	3.02	0.975	5.61	0.0546	0.0184

* Fasted without access to stools.

promptly. By virtue of this compensating process the total plasma protein decreases in a linear manner without abrupt swings, a finding of striking interest.

Why the protein concentration should remain high in the face of a perfectly steady fall of total protein is obscure. It may illustrate the natural attempt at homeostasis. The albumin-globulin ratio is seen to fall quickly and to remain more or less constant throughout the fasting period. This may perhaps be correlated with the similar changes in plasma volume. If albumin in large amounts is lost early, then the loss of osmotic pressure

concentrates the blood keeping the protein concentration constant and masking the real fall of total protein.

In men who have been partially starved for two days there is a loss of total protein, but no change of the albumin-globulin ratio (3). Longer fasting may result in a relative decrease in albumin as is seen in malnutrition edema. In rats, even in two days the loss of albumin is manifest. Presumably, then, two days of fasting in rats may represent a much longer time in man, and disclose both the general loss of all protein and the specific loss of albumin.

TABLE 4
Albumin and globulin and albumin-globulin ratios in starved rats

DAYS STARVED	NUMBER OF RATS	SPECIMEN I			SPECIMEN II			SPECIMEN III		
		Albumin	Globulin	Ratio	Albumin	Globulin	Ratio	Albumin	Globulin	Ratio
Large rats										
0	2	3.27	3.61	0.90:1	0.145	0.265	0.55:1			
6	4	2.00	4.95	0.40:1	0.103	0.217	0.48:1			
10	3	3.01	3.11	0.97:1						
14	4	2.03	4.11	0.50:1	0.089	0.166	0.53:1			
Small rats										
0	16	2.98	3.35	0.91:1	0.162	0.167	1.03:1	0.020*	0.054	0.43:1
2	5	2.48	4.22	0.59:1	0.115	0.159	0.72:1	0.029	0.021	1.37:1
2†	5	2.52	3.31	0.76:1	0.124	0.184	0.67:1	0.032	0.033	0.97:1
5	4	3.67	2.59	1.43:1	0.153	0.155	0.99:1			
7	5	2.74	3.74	0.73:1	0.108	0.185	0.57:1	0.023	0.040	0.57:1
8	4	2.10	3.56	0.59:1	0.151	9.109	1.37:1			
11	3	2.02	3.84	0.52:1	0.115	0.121	0.95:1			
13	4	2.36	3.25	0.72:1	0.086	0.123	0.69:1	0.015	0.034	0.44:1

* Two rats only.

† Fasted without access to feces.

We have evidence, therefore, that the protein concentration remains at normal levels for nearly a week in the fasting rat, which may correspond to several months of inadequate diet in man. Nevertheless, even though the concentration remains unchanged there is a steady fall in total protein which eventually in the rat, and presumably also analogously in man, makes maintenance of the normal concentration impossible. Then with the loss in compensation clinical signs of hypoproteinemia might be expected to occur in man, although not noted in the rat.

2. *Tissue space protein:* Speculation concerning tissue fluid protein changes is even more hazardous than that concerning plasma proteins.

It is obvious that to the true plasma protein a considerable amount of other protein is added during the perfusion. Roughly, both specimens II and III contain about twice as much protein as a similar dilution of blood. This excess protein is presumably washed into the blood stream during the perfusion from the lymph spaces of the body rather than mobilized from such depots as the liver, or even quickly manufactured there. This quantity of protein is apparently not reduced greatly by fasting but since the albumin of the dilute specimens falls roughly to the same degree as does that of the undiluted specimen, it seems likely that the albumin fraction of the lymph protein decreases commensurably with that of the plasma protein.

There is some evidence to show that normally (4) (5) lymph has about the same albumin-globulin ratio as plasma. The fact that the albumin-globulin ratio of the perfused specimen (specimen II) which quantitatively contained lymph protein fell during starvation to about the same degree as undiluted plasma supports the argument that lymph contains albumin and globulin in the usual plasma proportions. The actual albumin-globulin ratios were not definitely higher in the perfused specimens than in the original plasma. This, then, means that the pooled lymph protein of these experiments has about the same composition as plasma protein, although, as Drinker suggests, lymph formed in different locations may vary widely.

Finally, the tissue-fluid proteins are seen to enter intimately into the same changes undergone by the plasma proteins during the stress of starvation, and therefore possibly also during normal life.

CONCLUSIONS

1. A perfusion method for determining total blood volume of rats is described.
2. By the use of this method and an estimation of the concentration of protein in the blood, the total plasma protein of a rat may be measured.
3. During fasting the total plasma protein decrease is proportional to time.
4. The protein of lymph resembles that of plasma in its composition and its probable entrance into the same bodily activities.

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THE EFFECT OF DIFFERENT PER CENTS OF PROTEIN IN THE DIET ON BACHELOR AND VIRGIN RATS¹

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We have previously published (Slonaker, 1931 a, b, c, d, e, f, g) the results of different per cents of protein in the diet on the original matings. These pairs were allowed to breed and reproduce in a normal manner. Since breeding, as we have previously shown (Slonaker, 1927), influences body weight, spontaneous activity and possibly the span of life, we made another test to determine the effect of similar diets on strict bachelor and virgin rats. As in the former experiment these animals were segregated into five groups, I, II, III, IV and V, and were fed synthetic diets containing 10.3, 14.2, 18.2, 22.2 and 26.3 per cent protein respectively. Details of the diet and procedure have been published (Slonaker, 1931a).

The number of revolving cages available limited the number of animals under this observation to four bachelors in each group and six virgins in group I and ten virgins in each of the remaining groups. The animals composing each of these groups and their ancestors had been previously fed the same diets respectively. These animals were kept in their respective cages during their life span. This arrangement enabled us to ascertain the effect of these diets on sexual maturity, sexual life span, growth, spontaneous activity, and the life span unaffected by the variable factors incident to breeding and rearing young.

Activity. The results are summarized in table 1. Owing to the fact that male rats are usually slow in learning to turn their cages and that some never acquire the art we found that the number of males used in each group was insufficient to give a true average. Furthermore we have recently shown (Slonaker, 1935) that the activity of males closely adjacent to mature females was greatly increased due to the sex-drive stimulus of the females. Since groups I, II and one of group III were in close proximity to females their records were of no value in showing the effect of these diets

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on activity because of the additional sex-drive stimulus. This was especially noticeable in group III. The one male closely adjacent to females averaged 4537 revolutions daily while the remaining males, remote from females, averaged 625 revolutions daily throughout the life span. The effect of these diets on the activity of the males will therefore not be considered.

In this former paper (Slonaker, 1931b) we made a computed allowance in the females for loss of activity due to reproduction. Comparing our present results with the former we find that our computed allowance for reproduction was well within the limit of safety. The total computed miles run for each group was: I, 3803; II, 4133; III, 4097; IV, 3197; V, 3110. Converting the total revolutions of these virgin rats into miles we have I, 3469; II, 5104; III, 5041; IV, 4691; and V, 3082. With the exception of group I this is the same order of efficiency as shown in the

TABLE 1
Average activity of each group of females as indicated by the revolutions of the cages

GROUP	PROTEIN	NUMBER	AVERAGE DAYS IN CAGES	AVERAGE REVOLUTIONS AND MILES RUN			
				Revolutions		Miles	
				Total	Daily	Total	Daily
<i>per cent</i>							
I	10.3	6	905 \pm 18.3	3885106	4293 \pm 217	3469	3.83
II	14.2	10	952 \pm 11.9	5717530	6006 \pm 126	5104	5.36
III	18.2	10	869 \pm 8.6	5646925	6500 \pm 124	5041	5.80
IV	22.2	9	963 \pm 9.0	5153549	5351 \pm 133	4691	4.78
V	26.3	10	852 \pm 6.1	4258475	4998 \pm 58	3802	4.46

former experiment. The average daily run also corresponds, with the exception of group I. The reversal of the order of efficiency in total and daily activity in groups II and III was due to the difference in the number of days in the cages. The older rats, being less active, reduced the average daily run. This accounts for the lower average daily run in groups I, II and IV. The average activity of these groups at the same ages given for groups III and V in table 1 were 4329, 6505 and 5227 respectively. The lowest average shown by group I was due to one of the females being very inactive. During its life span it ran less than half the number of revolutions the others ran. This was apparently due to some abnormality in this animal. If this animal were eliminated the average run of the remaining five would be 5201 revolutions daily and a total of 46,032,111 revolutions for their whole life span. In regard to the efficiency of these diets on these virgin rats the order, from greatest to least, was groups II, III, IV, I and V. In the original mating the order was the same except group I preceded group IV.

Growth. In tables 2, 3 and 4 we have given the average ages and weights of each group at the beginning of the experiment, at maximum weight, and at death. Two of the females in group II and one in group III developed tumors at about the age of 700 days. These were the only animals in both sexes in which tumors were found. These tumors greatly increased the maximum and death weights of these animals from their appearance to death. Since this was an abnormal condition the weights

TABLE 2
Average ages and weights of rats at beginning of experiment

GROUP	PROTEIN	MALES						FEMALES					
		Num- ber	Age		Weight		Num- ber	Age		Weight		Aver- age	P.E.
			Average	Range	Average	P.E.		Average	Range	Aver- age	P.E.		
<i>per cent</i>													
I	10.3	4	77	74- 81	105	±4.7	6	46	38- 62	66	±3.6		
II	14.2	4	69	69- 69	81	±1.6	10	30	27- 35	35	±0.8		
III	18.2	4	76	76- 76	99	±1.0	10	57	64-140	96	±1.6		
IV	22.2	4	42	42- 42	58	±0.8	10	33	36- 62	44	±0.7		
V	26.3	4	111	111-111	169	±3.6	10	56	52- 65	97	±0.6		

TABLE 3
Average ages at maximum weight and average maximum weights of each group

GROUP	PROTEIN	MALES						FEMALES					
		Num- ber	Age		Weight		Num- ber	Age		Weight		Aver- age	P.E.
			Average	Range	Average	P.E.		Average	Range	Aver- age	P.E.		
<i>per cent</i>													
I	10.3	4	608	455-839	347	±8.4	6	758	501- 989	245	±2.3		
II	14.2	4	674	476-871	306	±5.0	8	759	460- 980	244	±3.1		
III	18.2	4	677	530-877	304	±2.4	9	824	573-1025	296	±3.2		
IV	22.2	4	708	358-876	325	±4.6	10	803	555-1105	247	±1.8		
V	26.3	4	416	458-580	328	±5.1	10	659	497- 881	252	±2.2		

of these tumor rats were not used in computing the average maximum and death weights given in tables 3 and 4.

All of these animals were taken from the first, second and third generations of the original mating (Slonaker, 1931a). At the beginning of the experiment the average ages were older than those of the original pairs and yet the average weights, as given in table 2, were somewhat less. This may have been due to the original animals having been reared on a different diet until the beginning of that experiment.

Table 3 shows that the average maximum weights of the groups of males did not conform to the order found in the original pairs. This was probably due to the small number in each group. While these weights do not show any marked effect of diet they do show that the average weight of all groups of bachelors (322 grams) was greater than the average weights of all the original mated males (298 grams). This substantiates our previous results that bachelors usually attain a greater weight than mated males (Slonaker, 1927). This table also shows that the females of group III surpassed all others in maximum weight and that the remaining groups were nearly the same. In the original matings group II, followed closely by group III, was the heaviest. The females reached their maximum weight at a considerably older age than the males. This verifies the results found in the original matings. Both sexes reached their maximum weights at later ages than those of the original matings. Owing to

TABLE 4
Average ages and weights at death

GROUP	PROTEIN	MALES					FEMALES				
		Num- ber	Age		Weight		Num- ber	Age		Weight	
			Aver- age	Range	Aver- age	P.E.		Aver- age	Range	Aver- age	P.E.
<i>per cent</i>											
I	10.3	4	996	642-1161	175	±1.4	6	992	622-1195	162	±3.1
II	14.2	4	956	499-1203	188	±2.3	8	968	771-1152	174	±2.3
III	18.2	4	1077	815-1222	166	±3.3	9	918	703-1094	204	±4.2
IV	22.2	4	1012	864-1141	215	±3.3	10	979	761-1189	165	±2.1
V	26.3	4	944	671-1137	201	±2.3	10	900	700-1010	188	±2.6

the small number of animals used no definite conclusions can be made in regard to the effect of these diets on maximum weight.

The average weights at death, with four exceptions, were less than the corresponding groups and sexes of the original matings. These four exceptions were the males of group IV and the females of groups I, III and V. These exceptions may have been due to the small number of animals in the groups. This lower average weight at death was mainly due to the much greater age at death of these animals. The average age at death of the bachelors was 258 days and of the virgins 166 days greater than that of the corresponding sexes of the original matings. Due to these marked differences in ages of death and the effect of senility on body weight the order of the effect on weight in these bachelors and virgins does not conform to that of the original pairs. At the same ages the order was practically the same. It is noted, however, that both sexes of group V, like the original matings, had the shortest life span.

Sexual maturity and sexual span. It has been proved both by vaginal smears and mating tests that the rhythmic peaks in the activity curve of a female rat correspond to and are synchronous with its estrual periods. Using this fact and the individual activity curves we were able to determine the age at which oestruation stopped for each female and thus determine its sexual span. Since the male activity curve gives no clue regarding sexual phenomena we did not ascertain the sexual span in this sex. Table 5 gives the average results of each group of females. The age at which the vagina opened was gotten by daily observations. The ages at which estruation ceased were procured from the activity curves. We have assumed that the opening of the vagina indicates sexual maturity. We have found that successful mating in other females has taken place as soon as four days after this event. The average age at the opening of the vagina in the different groups varied from 50 days in groups II and IV

TABLE 5

Average length in days of sexual life of females as determined by observations and activity curves

GROUP	PROTEIN	NUMBER	OPENING OF VAGINA		LAST REGULAR CYCLE		LAST CYCLE		SEXUAL SPAN	
			Age	Range	Age	Range	Age	Range	Days	Range
<i>per cent</i>										
I	10.3	6	56 ± 1.32	48-73	419	330-512	694	536-874	639	463-826
II	14.2	10	50 ± 0.27	46-59	444	285-587	722	601-866	672	554-817
III	18.2	10	66 ± 0.60	59-82	370	222-479	676	600-827	619	446-756
IV	22.2	10	50 ± 0.41	43-66	395	292-554	718	542-841	668	476-793
V	26.3	10	66 ± 0.31	62-75	335	205-494	695	474-886	632	409-824

to 66 days in groups III and V. This does not indicate any special effect of one diet over another. Observations now in progress on several hundred animals will give more definite information. The fact that the earliest age in group V was 62 days, which was older than the first appearance in any of the other groups, suggests a greater retarding effect on pubescence by the high protein diet.

The activity curves showed that the cessation of estruation exhibited marked individual variations. After the regular cycles had stopped some showed many days during which no estruation occurred followed by regular cycles of from two to several in number. In others only single or double periods were found with no regular interval intervening. The final termination in most all cases was marked by these single cycles. These results correspond closely with our former observations (Slonaker, 1924). The age of pubescence as determined by the opening of the vagina in these virgins is earlier than that formerly ascertained by mating.

Table 5 shows that the regular cycles stopped at an earlier age in group V and persisted longer in group II. It also shows that the average sexual span was longest in group II, but the differences are not very significant. The last cycle is difficult to determine accurately because of the general reduction of activity and the sharpness of the estrual peaks with advancing age. These last cycles may not have resulted in productivity had mating tests been made, but that at least some of them would have been successful is indicated by the age of a large number of mothers which have delivered litters when over 600 days of age. We have separate records of young being delivered when the mothers were 772, 788 and 794 days of age. The sexual span in all the groups, as determined by the above method, was considerably longer than that of the original matings in which the ages of the mothers at the first and last litters born were used in computing the duration of fertility. This suggests that in mated animals many estruations may occur which are not productive of young. The longest sexual

TABLE 6
Average life span in days

GROUP	PROTEIN	MALES					FEMALES				
		Num- ber	Days	P.E.	Range	Living 1000 days	Num- ber	Days	P.E.	Range	Living 1000 days
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
I	10.3	4	996	± 34.65	642-1161	75	6	992	± 21.72	622-1195	67
II	14.2	4	956	± 47.15	499-1203	50	10	999	± 8.54	771-1152	60
III	18.2	4	1077	± 27.31	815-1222	75	10	927	± 8.70	703-1094	40
IV	22.2	4	1012	± 16.79	864-1141	50	10	978	± 9.39	761-1189	50
V	26.3	4	944	± 28.65	671-1137	50	10	900	± 6.12	700-1010	10

span was found in group II, which is in accord with the results of the original matings. The general results, however, do not show any notable or consistent effect of these diets on the sexual span in these virgins.

Life span. A summary of the results on the average life span is given in table 6. Both sexes in each group had individuals which reached a very old age. The oldest age attained was by a male in group III and this group had the greatest average age. Each of the groups had individuals in both sexes which reached an age of 1000 days or more. The percentage of each sex in each group which lived to 1000 or more days is given in table 6. A male in group II died at the early age of 499 days from lung infection. This was replaced by a new male which died at the age of 1000 days. If this latter animal had by chance been selected instead of the original one the percentage of this group reaching 1000 days would have been the same as in groups I and II. This again shows that the number in each group was too small to give true average results. These results, however, do

indicate that the higher per cents of protein in the diet have a marked effect by shortening the life span in both sexes. This is in accordance with our previous findings with the original matings.

SUMMARY

1. It is shown that, on account of individual variations, the number of animals used in each group should have been greater to give true average results.

2. The order of efficiency of these diets as determined by the total distance run was, from greatest to least, II 14.2 per cent, 5104; III 18.2 per cent, 5041; IV 22.2 per cent, 4691; V 26.3 per cent, 3802; and I 10.3 per cent, 3461 miles.

3. Both sexes reached their maximum weight at later ages than mated animals. The results on maximum and death weights were not significant.

4. The duration of sexual life was greatest in group II 14.2 per cent and least in groups I 10.2 per cent and V 26.3 per cent. The sexual span was longer and the age at which estruation ceased was greater than has been determined by breeding.

5. The life span of both sexes was shortest in group V. It was longest in the males of group III 18.2 per cent and the females of group II 14.2 per cent.

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THE EFFECT OF REDUCED ATMOSPHERIC PRESSURE ON THE LEUKOCYTE COUNT¹

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Despite the uniformity of results obtained by many investigators concerning the erythrocyte and hemoglobin responses to reduced oxygen tension, there have been relatively few studies made of the leukocytes under similar conditions, and the reports (1-11) that have been made are not concordant.

During the course of other studies upon rats kept in a reduced pressure chamber, striking changes in the total leukocyte counts were noted. The present communication deals, therefore, with the total and differential leukocyte counts as influenced by a reduction in barometric pressure.

METHODS. The reduced pressure chamber described by Loevenhart and his associates (12) was used in this study. It is possible to rapidly lower the pressure in this chamber to any desired level where it is automatically maintained within 2 to 3 mm. Hg variation. The ventilation rate through the chamber is 22 to 24 liters per minute which prevents a rise of carbon dioxide above 0.3 per cent when using a maximum number of animals (12 rats or 8 guinea pigs). It is only necessary to open the chamber once a week since enough food (Steenbock ration for rats) is placed in the chamber at one time to last for this period and drinking water is aspirated into the chamber through a flexible copper tube.

Mature male white rats weighing 290 to 450 grams were used in the majority of the experiments. Results in female rats were found to be comparable to those in males. Experiments were done on splenectomized rats in order to compare the possible effect of an absence of this reservoir.

The animals were constantly exposed to the reduced pressure except for periods of 3 to 4 hours when counts were being made and on rare occasions for a few minutes when repair of the apparatus was necessary.

Erythrocyte and reticulocyte counts and hemoglobin determinations were made to establish the validity of the experimental procedure and to furnish data which might enable us to compare the results with those of others. In the early experiments, the Sahli hemoglobinometer was used,

¹ Supported in part by a grant from the Wisconsin Alumni Research Foundation.

in later experiments the Haden-Hausser, comparative standards being used throughout. Counts were made by the usual methods using pipettes certified by the U. S. Bureau of Standards. All erythrocyte counts were done in duplicate usually from a single pipette, all leukocyte counts from paired pipettes, in duplicate or quadruplicate. Blood was secured in rats by cutting a tail vein and in guinea pigs by puncturing an ear vein. Considerable care was exercised to keep blood loss at a minimum. The differential counts were complete and of 200 cells stained by Wright's method. Only the neutrophiles are reported because they are most easily identified. The lymphocytes and monocytes in the rat are differentiated from each other with difficulty. The reticulocyte percentages were obtained from examination of 1000 erythrocytes, stained with Cresyl blue. The average deviation from the mean for leukocyte counts in duplicate was found to be ± 3 per cent and for erythrocyte counts ± 2 per cent.

EXPERIMENTS. Complete blood counts were done on 10 male white rats. Five of these were splenectomized on the following day. Blood counts were again made one week later and the rats were placed in the chamber on the following day. (In all subsequent experiments counts were done on the day the animals were placed in the chamber.) The pressure was reduced, within an hour, to 422 mm. Hg. For the first two to three days in the chamber the rats were depressed, ate little and were dyspneic; subsequently they were active, ate well and showed no respiratory embarrassment. The maximum change in weight observed after one week was a loss of 25 grams in a 350 gram rat. In subsequent experiments minimal weight changes were also the rule. The results in this preliminary experiment of seven days' duration showed a significant decrease in the total number of leukocytes, the decrease occurring in eight of the ten animals, failing to occur in one splenectomized and one normal.

One month after the completion of this experiment the leukocytes as well as the other blood components of these ten rats had returned to the control level. The return of the leukocytes to normal has been found to occur usually in less than two weeks, but in this group earlier counts were not made. The experiment was now repeated with the same animals under similar conditions but for a period of four weeks. The animals were placed in the chamber on the day of the control counts and blood counts were done at weekly intervals thereafter. The results of the leukocyte counts for this experiment are shown in table 1.

A diminution in the number of leukocytes was noted in every case and this decrease was persistent at each weekly count except in one animal, which, in the second week, showed an increase of 1900 leukocytes over the control count. This increase was abolished the next week and the count was thereafter below the control level.

The percentage increase in neutrophiles was not as regular as the de-

crease in total leukocytes but occurred in the majority of the animals. No significance is attached to these changes because they are slight. The total number of lymphocytes was decreased. Subsequent experiments were conducted to check these findings further, using six splenectomized and four normal rats. Counts were made prior to and six and twelve days after the animals had been kept at a barometric pressure of 422 mm.

TABLE 1

Averages of the leukocyte counts of ten rats (5 splenectomized) exposed for four weeks to a barometric pressure of 422 mm. Hg, partial pressure of oxygen 86 mm. Hg, equivalent oxygen tension 12 per cent of an atmosphere, corresponding altitude approximately 16,000 feet

	DATE	TOTAL LEUKOCYTE COUNTS			NEUTROPHILES
		Average of 10	Average decrease	Per cent decrease	
Control.....	3/23	18,568			25.9
Experimental period.....	3/30	14,098	-4470	24	28.5
Experimental period.....	4/6	14,746	-3822	20	33.4
Experimental period.....	4/13	13,460	-5108	28	29.6
Experimental period.....	4/20	13,824	-4744	26	29.8
Control.....	4/26	18,953			

TABLE 2

The maximum, minimum and average values of the leukocyte counts of 12 normal and 14 splenectomized rats kept at a barometric pressure of 422 mm. Hg for 6 to 8 days

		CONTROL COUNTS			6 TO 8 DAYS LATER		
		W.B.C. per cu. mm.	Neutrophiles		W.B.C. per cu. mm.	Neutrophiles	
			Per cent of total	Total		Per cent of total	Total
Normal rats (12)	Maximum.....	18,125	39.0	5,972	16,300	39.0	5,864
	Minimum.....	11,225	17.0	2,775	6,300	21.5	2,508
	Average.....	14,645	27.3	3,966	10,864	32.3	3,534
Splenectomized rats (14)	Maximum.....	33,675	50.0	9,492	24,675	48.5	8,019
	Minimum.....	13,700	17.0	2,625	11,750	18.0	2,655
	Average.....	22,453	29.9	6,515	17,725	29.6	5,296

Hg. The results confirmed those of the previous experiments, the average decrease in leukocytes in the splenectomized rats being 4554 and in the normals, 4991.

Table 2 summarizes the results of all experiments in which leukocyte counts were made on normal and splenectomized animals after being kept at a barometric pressure of 422 mm. Hg for six to eight days. Leukope-

nia occurred in 11 of the 12 normal, and 12 of the 14 splenectomized rats. There was lack of a consistent relative or absolute change in the neutrophiles in this group of animals. The comparatively high level of the original leukocyte counts of the splenectomized animals is to be noted.

Leukocytosis has often been reported during exposure to a reduced atmospheric pressure. The question arose as to whether leukocytosis was occurring in these animals during the first hours or days, since up to this time, counts had not been made until after six days of exposure. To determine this point, nine normal rats were exposed to a barometric pressure of 282 mm. Hg and counts were made prior to and twenty hours after reduction of the pressure. One rat which died within 20 hours was excluded, and the results shown in table 3 are those of the remaining eight.

Each of these animals showed a considerable leukocytosis, the smallest increase being 4637 cells. A percentage increase in neutrophiles was also

TABLE 3

The effect of reduced atmospheric pressure upon the leukocyte counts of eight rats

Duration of experiment 20 hours. Barometric pressure 282 mm. Hg, partial pressure of oxygen 57 mm. Hg, corresponding oxygen tension, 8 per cent of an atmosphere, equivalent altitude approximately 25,000 feet.

RATS (8)	CONTROL COUNT 5/28	COUNT 5/29	CHANGE
Average total W.B.C. per cu. mm.	12,889	22,564	+9675
Per cent neutrophiles	39.8	66.9	+27.1

invariable, averaging 27 per cent. Similar results will be noted during the first period shown in figure 1 at a pressure of 422 mm. Hg. Here the leukocytosis is less marked but equally consistent.

Eight guinea pigs kept at a pressure of 422 mm. Hg for 42 hours exhibited leukocytosis in every instance, increasing from an average control level of 13,230 to 17,838 per cu. mm. Experiments of longer duration in guinea pigs showed a trend similar to that of rats though the leukopenia was less marked and less regular. These experiments of longer duration on the guinea pig are, however, not conclusive as they were done during hot weather and the temperature within the chamber was usually 30°C. or higher, whereas the rat experiments were conducted at temperatures ranging between 23° and 27°C.

To complete the study, rats were acclimatized gradually by reducing the pressure from 740 to 282 mm. Hg in weekly stages. The animals were exposed successively to three levels of 422, 352 and 282 mm. Hg. Complete blood counts were made after the rats had been at a given pres-

sure for one week and on the day following each reduction. The results for the total leukocytes, total neutrophiles, and percentage of neutrophiles are graphically shown in the figure. These figures represent the average of results from eight animals.

Leukocytosis with an increase in neutrophiles will be seen to occur within 24 hours at a pressure of 422 mm. Hg. At the end of one week

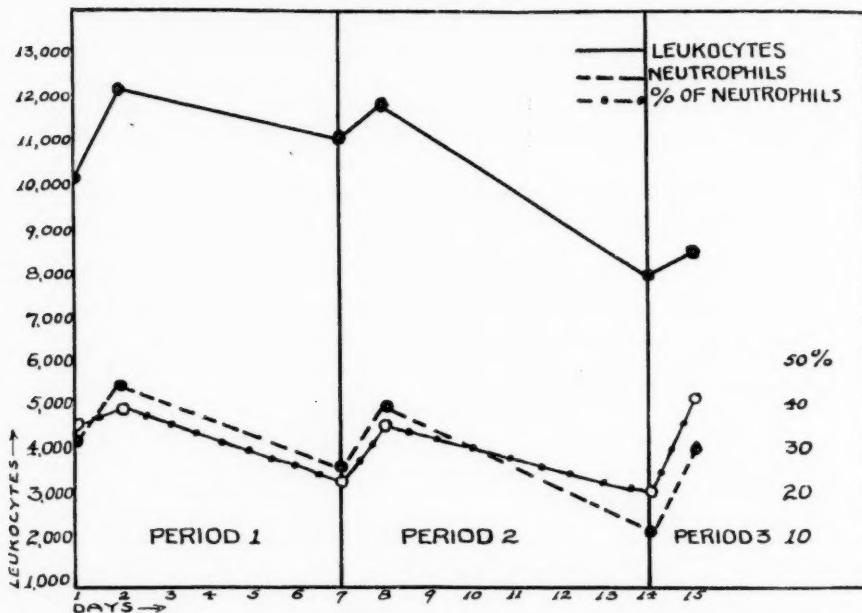


Fig. 1. The response of the leukocytes and neutrophiles to a further reduction of barometric pressure after remaining at a constant level of diminished oxygen tension for a week. Period 1: Barometric pressure 422 mm. Hg, partial pressure of oxygen 86 mm. Hg, equivalent oxygen tension 12 per cent of an atmosphere, approximate altitude 16,000 feet. Period 2: Barometric pressure 352 mm. Hg, partial pressure of oxygen 72 mm. Hg, equivalent oxygen tension 10 per cent of an atmosphere, approximate altitude 20,000 feet. Period 3: Barometric pressure 282 mm. Hg, partial pressure of oxygen 57 mm. Hg, equivalent oxygen tension 8 per cent of an atmosphere, approximate altitude 25,000 feet.

the total number of leukocytes is decreased, though not to the control level. The neutrophiles, relatively and absolutely have fallen below the control level, so that a relative lymphocytosis exists. Upon returning the animals to the chamber and reducing the pressure to 352 mm. Hg, another increase in leukocytes and neutrophiles follows within 24 hours, only to be succeeded at the end of the second week by distinct leukopenia and

neutropenia. (Neutropenia here occurred in each of the eight animals.) When the pressure was further reduced to 282 mm. Hg an elevation in the total leukocyte count again occurred although it did not reach the control level. The failure of the total count to reach the original height results from the lymphopenia since the neutrophiles approximate the control level. The plan to carry these experiments further was impossible because of death of the rats on the day following the last counts due to an accidental decrease in pressure.

To serve as a standard for comparison, the average increase in hemoglobin, erythrocytes, and reticulocytes that occurred in this experiment of two weeks' duration, are shown below. These results are typical for all the experiments of similar duration and under similar pressure conditions. Other indications of increased generation such as the appearance of more nucleated and polychromatophilic cells in the blood smears have been noted regularly.

	HEMOGLOBIN*		ERYTHROCYTES	RETICULOCYTES per cent
	grams	per cent		
June 7 Control	12.7	82.6	8,819,000	1.1
8	13.7	89.1	9,142,000	1.2
13	15.7	101.8	9,611,000	3.8
14	Not determined			3.8
20	18	116.3	10,675,000	6.9

* Haden-Hausser hemoglobinometer—15.4 grams hemoglobin per 100 cc. = 100 per cent.

It has been observed in unpublished experiments, in which the diminished pressure was maintained at a constant level for four weeks, that a maximum increase in hemoglobin, erythrocytes and reticulocytes is attained by the end of the second or third week. Thereafter the hemoglobin and erythrocytes remain stationary whereas the reticulocytes recede toward the control level.

It will be noted that the average increase in hemoglobin over the basal level is roughly twice that of the percentage increase in erythrocytes. This result is similar to that of Dallwig, Kolls and Loevenhart (12) in most of their experiments on other animals. Confirmation is further obtained by examination of the blood smears. The majority of the individual erythrocytes have a diameter equal to, or greater than, the normal maximum size and contain more hemoglobin than during the control period. This latter observation has been made previously by Dubin (13) who noted a considerable increase in spread for the diameters of erythrocytes over the normal, with an increase in the number of cells of greater diameter.

DISCUSSION. In the above experiments the consistent finding of leukocytosis with a relative and absolute neutrophile increase followed in a few days by leukopenia, in rats kept at a low oxygen tension, appears to be of significance, particularly in view of the unfailing return to the control level upon removal to atmospheric pressure. Contrary to some reports, neutropenia in association with leukopenia was not invariable, though occasionally observed. No consistent variation in the differential blood picture occurred. It is appreciated that these experiments or any experiments in a chamber are not strictly comparable to those conducted at corresponding mountain altitudes since extraneous factors, especially the additional solar radiation, modify the differential blood picture in some degree (14).

It is believed that these findings clarify to a certain extent the apparently discordant results in the literature the obvious deduction being, that either leukocytosis or leukopenia may be obtained in a given experiment, depending entirely upon the length of exposure to a reduced barometric pressure.

The longest experiments reported here in detail are of four weeks' duration, during which time the leukopenia persisted. Whether this leukopenia would be permanent under the continued circumstance of reduced pressure is not as yet known.

The mechanisms involved in producing the changes in the number of leukocytes in the peripheral blood are not entirely clear. Concentration of the blood, bone marrow stimulation, or an expulsion of cells from various reservoirs resulting from smooth muscle contraction as shown by De Boer and Carroll (15) and Barcroft (16) for erythrocytes, may all be invoked to explain the early increase in leukocytes. An explanation for the subsequent leukopenia is not obvious. Four possibilities are suggested:

a. A redistribution of cells and no true leukopenia occurs. The degree of leukopenia, the changes in the differential count, and the fact that sampling was done from a large vein with free flow of blood suggest that such is not the case.

b. Obliteration of leukogenetic tissue by the hyperplastic erythropoietic tissue. Examination of the bone marrow² after one week's exposure failed to give evidence substantiating such an hypothesis.

c. Increased destruction of leukocytes. No increase in the number of degenerate or broken cells were found in the blood smears.

d. Depression of the functional activity of leukogenetic tissue in the bone marrow and lymph nodes. While this is seemingly the most probable explanation, it has not yet been possible to establish this premise.

² Dr. C. H. Bunting examined some of the bone marrow sections and we wish gratefully to acknowledge his aid.

It is interesting to speculate upon the results of these experiments in which presumably a single factor, i.e., a reduction in barometric pressure, elicits such a striking contrast in its effect upon different components of the blood, bringing about regular increases in the erythrocytes and decreases in the leukocytes associated with hyperplasia of the erythropoietic tissue without concomitant hyperplasia of the leukopoietic tissue. No similar situation has been established in which polycythemia with leukopenia occurs regularly though the opposite state of leukocytosis and anemia is common, most strikingly in leukemia.

SUMMARY

Normal rats kept at a reduced atmospheric pressure in a chamber, developed early leukocytosis followed by a persistent leukopenia with a concomitant increase in hemoglobin, erythrocytes and reticulocytes. The changes in the differential leukocyte formula were not consistent. Relative neutropenia did not occur regularly except in the animals which were exposed to successive reductions in barometric pressure. The increase in hemoglobin from the control level was disproportionately greater than the increase in erythrocytes. The individual erythrocytes tended to be larger and better filled with hemoglobin.

Results in splenectomized rats were similar in type and degree to those in normal rats. The guinea pig appears to respond in much the same manner as the rat.

It is suggested that the temporary leukocytosis following a reduction in barometric pressure is principally due to the emptying of reservoirs of cells such as the spleen, liver and lungs whereas the subsequent leukopenia results from a functional depression of the bone marrow and lymph nodes which prevents a delivery of leukocytes to the circulating blood.

The differences in the leukocytic responses to reduced atmospheric pressure, i.e., early leukocytosis and later leukopenia appear to be due solely to the element of time. This factor of acclimatization may well explain the discordant results of some of the previous observers.

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THE EFFECT OF CHOLECYSTOKININ ON THE CHOLEDODOCHO-DUODENAL MECHANISM (SPHINCTER OF ODDI)

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Since the evacuation of the gall bladder is dependent on the resistance of the choledoco-duodenal mechanism (intramural resistance), it was considered important to study the effect of cholecystokinin on that mechanism (1). We were primarily interested in ascertaining whether the hormone increased or decreased the intramural resistance by acting directly on the duodenal or sphincteric musculature, rather than in determining whether a reflex connection between the gall bladder and sphincter of Oddi exists. The latter question had to be considered, however, because it is possible that the contracting gall bladder may initiate a reflex relaxation of the sphincter.

METHODS. Since the resistance offered by the choledoco-duodenal mechanism is apparently dependent on a special sphincter (Oddi) and the "tone" and motility of the duodenum, simultaneous records were made of duodenal motility and the intramural resistance. Records of intragall-bladder pressure were also made. To record intragall-bladder pressure the method of Ivy and Oldberg (2) was used. To make simultaneous records of duodenal motility and intramural resistance the method of Lueth (3) was employed. The type of pressure regulatory apparatus, including the electric drop recorder of the rate of inflow, is shown in figure 1. This method, of course, renders it possible to determine manometrically in millimeters of Ringer's solution (39°C.) the pressure required to overcome the intramural resistance as well as variations in the rate of inflow from the common duct into the duodenum. The method for determining the inflow pressure is sensitive within 10 mm. The location of the balloon in the duodenum and its relative fixation is easily controlled.

The dogs used were anesthetised with barbital, ether being used during the operative procedure. A record of blood pressure was made routinely to assure us that the general condition of the subject was good.

The threshold dose of the cholecystokinin preparation used was 1 mgm. per dog. From 5 to 10 mgm. were injected. This preparation of the

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hormone, as will be noted from our results, was apparently different from that used by Lueth, Ivy and Kloster (4) in that it rather uniformly increased duodenal motility and tone. Their preparation had no effect on the intestine in four anesthetised dogs and had a variable effect on unanesthetised dogs. We have no trustworthy explanation to offer for this difference. The same preparation of hormone was used throughout these experiments.

RESULTS. Three groups of experiments were performed. In the *first group*, after a suitable control period the cholecystokinin was injected during a period of one or two minutes. This was done to determine the changes which resulted from the abrupt action of the hormone. In the

second group, the hormone was dissolved in a quantity of Ringer's solution and injected intravenously at a timed rate. This was done to simulate physiologic conditions more closely. In a *third group*, the hormone was injected twenty minutes or longer after cholecystectomy to determine whether the gall bladder might have influenced the results observed in the foregoing experiments.

Results of the experiments in groups one and two. In the first group eighteen dogs were injected twenty-seven times with doses of the hormone ranging from 5 to 10 mgm. In the second group six dogs were injected with doses ranging from 0.2 to 0.6 mgm. per minute for twenty minutes.

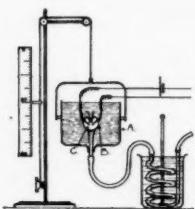
Since the results of these two groups of experiments were so similar they may be treated as a single group. The only difference in the results was that the reactions in the experiments of group one were more abrupt.

Fig. 1. Device for determining pressure, regulating and recording automatically the rate of "inflow" through the intramural portion of the common duct. An automatic electric heating device was placed in the vessel containing the thermometer.

The "control" intramural resistance observed in the twenty-four subjects ranged from 70 to 320 mm. of Ringer's solution pressure, the average being 215 mm.

Following the injection of cholecystokinin, an increase in duodenal motility was observed in 87 per cent of the tests (28 out of 33 tests). This increase in motility occurred after a latent period which closely approximated that of the gall-bladder contraction, namely, from fifteen seconds to one and a half minutes. The motility initiated was periodic in type. The first period of motility was longer than those following and usually lasted about four minutes. During this initial period a definite increase in duodenal "tone" was usually observed. A rather typical response is shown in graph 1.

The increase in duodenal motility and tone was associated with an in-



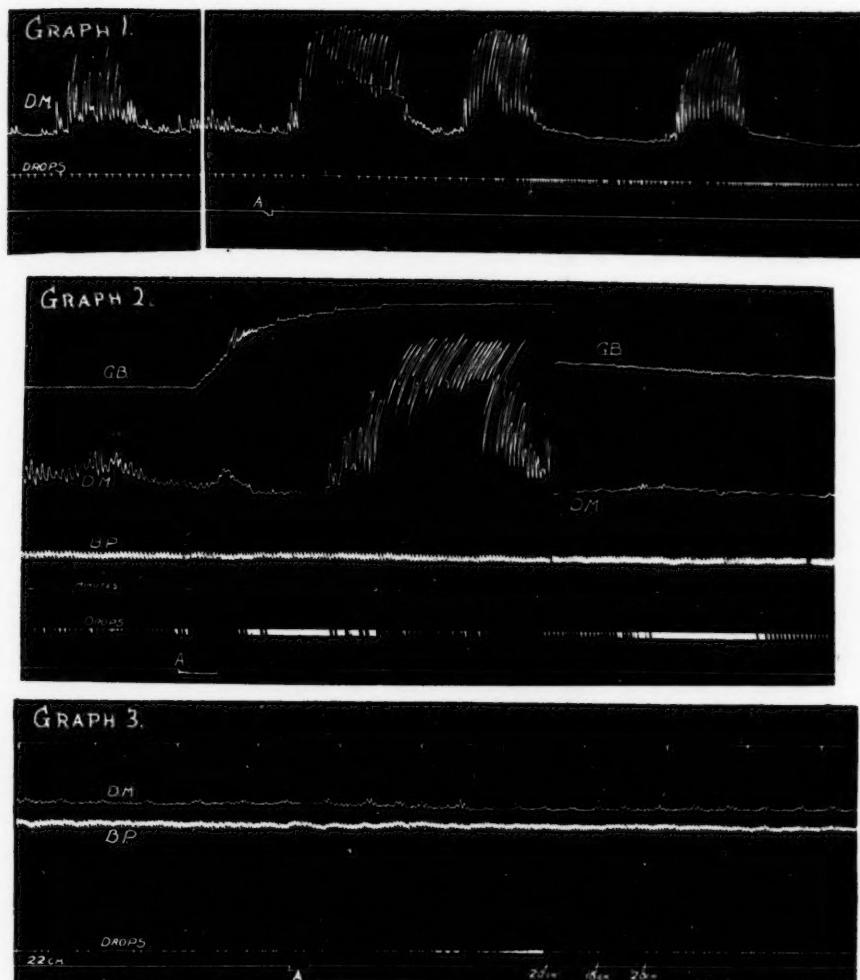


Fig. 2. G. B., gall-bladder pressure; B. P., blood pressure; D. M., duodenal motility; Drops, rate of flow through intramural portion of duct into duodenum.

Graph 1. At A an injection of cholecystokinin was made. Note periodic activity of duodenum and changes in intramural resistance.

Graph 2. At A an injection of cholecystokinin was made. For explanation see text.

Graph 3. At A an injection of cholecystokinin was made after cholecystectomy. Note changes in intramural resistance (drops) without much change in duodenal motility. At the points showing the notations 20, 18 and 20 cm. the intramural resistance was determined.

crease in intramural resistance in fifteen out of thirty-three tests. The maximum increase in resistance amounted to 100 mm. Following this initial period of increase in resistance, the resistance usually returned to the control level or was definitely decreased. This secondary decrease in intramural resistance amounted to from 20 to 50 mm., lasted a variable period of time, and occurred in sixteen out of thirty-three tests. The primary increase in resistance persisted in only two tests. In the other tests either no change or only an insignificant change occurred.

In graph 1 it is to be noted that during the period of increase in the tone of the duodenum, the inflow of fluid into the duodenum is definitely retarded; with a decrease in tone the inflow is definitely augmented. Although this was the rule it was not invariably true, nor was the increase in resistance always proportional to the recorded increase in duodenal tone. This confirms the observation of Lueth (3) that an increase in duodenal motility and tone as recorded by the balloon method is not always accompanied by an increase in intramural resistance.

The results observed in the following four experiments, we believe, are particularly significant in regard to the action of cholecystokinin on the common duct sphincter. In these four experiments, the latent period of the duodenal motor response was unusually long and the primary effect of cholecystokinin on intramural resistance resulted in a diminution of resistance. The maximum decrease of resistance observed in these four experiments amounted to 40 mm. of pressure (Ringer's). This occurred without appreciable change in the tone of the duodenum. It should be remembered in this connection that the balloon method records circular activity chiefly and that the longitudinal fibers of the duodenum may affect intramural resistance. But, we believe, the decrease in pressure was too great to be accounted for by a concealed change in the duodenal tone.

The results in graph 2 are particularly instructive in regard to the relation of duodenal "tone" to intramural resistance and the action of cholecystokinin. Shortly after the injection of the cholecystokinin the duodenum contracted slightly and the flow ceased. Then, the duodenum relaxed slightly and the flow was markedly increased. Following this, the duodenum manifested a marked increase in tone and motility for a period of four minutes which reduced the flow; but, regardless of the marked increase in tone of the duodenum, the flow did not cease! Then, (2nd part of the graph) after cessation of the duodenal motor response and with the tone of the duodenum at the control level, a marked periodic decrease in intramural resistance occurred. (The missing portion of the graph included four successively diminishing periods of activity and relative quiescence somewhat analogous to graph 1.) This would again indicate that the intramural resistance may at times be independent of duodenal tone and motility and that an independent sphincteric mechanism exists.

Results of the experiments in group three. It is established that the sphincter of the common duct becomes incompetent for a period after cholecystectomy (1). Puestow (5) using a special type of biliary fistula, in which it was possible to view the ampullar orifice of the duct, observed that the orifice was patulous twenty-four hours after cholecystectomy and that bile flowed through the orifice continuously instead of in spurts. Further, there is evidence (1) indicating that when the gall bladder contracts the intramural resistance decreases. McMaster and Elman (6) even observed such a phenomenon when both the cystic and common ducts were cut and cannulated. Thus, it is possible that our experimental procedure permits a possible reflex connection between the gall bladder and sphincter of function, which might influence the results observed in the above experiments. To determine this, it was necessary to remove the gall bladder and then, after a suitable control period, twenty or thirty minutes, inject cholecystokinin.

Four experiments were performed. After cholecystectomy the intramural resistance showed no change in two experiments; in one the resistance was definitely decreased, and in the other it was increased 20 mm. After injection of cholecystokinin duodenal motility was augmented in three instances and was unaffected in the other. In two instances the increase in motility was accomplished by a primary increase in intramural resistance; in the other the intramural resistance was unchanged. A secondary decrease (20 mm.) in intramural resistance occurred in two of the experiments. Thus, effects of the hormone were subject to the same variation as those noted in the experiments of group one and two. Graph 3 is a record obtained in one of these experiments. In that experiment it is to be noted that the hormone had but very little effect on duodenal motility; but the primary and secondary changes in intramural resistance are quite definite, and are associated with the slight changes in duodenal activity.

DISCUSSION. It is very difficult, if not impossible to separate directly and decisively the activity of the sphincter of Oddi from that of the duodenal musculature, because of their intimate anatomical relationship. The independent activity of the sphincter can be inferred only when changes in intramural resistance results without any change in the duodenum or when the changes are decidedly non-proportional. In our experiments when the intramural change in resistance was not concealed or complicated by a definitely detectable change in the duodenum, cholecystokinin decreased the intramural resistance. This strongly indicates that the unconcealed effect of cholecystokinin on the sphincter is inhibitory; though, because of the difficulties inherent in the interpretation of the results, we hesitate to draw such a definite and positive conclusion.

It is clear, however, that as a rule the changes in intramural resistance, which occur after the injection of cholecystokinin, are usually associated

with changes in duodenal "tone" and motility, and that these changes may occur independently of the gall bladder. This again emphasizes the importance of the rôle of duodenal tone in the evacuation of the gall bladder and the flow of bile into the intestine, since even the hormone—at least the preparation used in these experiments—affects duodenal tone and motility. *It should be pointed out that the observed increases in duodenal tone were sufficient to retard the evacuation of the gall bladder contracting under the influence of the hormone, but were not adequate to prevent it from evacuating.* However, in nine experiments, because of the high (plus 200 mm.) original or control intramural resistance, the gall bladder would have been unable to evacuate its contents during the initial period of heightened duodenal tone following the injection of cholecystokinin. It would have evacuated to some extent during the succeeding period of decrease in tone of the duodenum or secondary phase of decreased intramural resistance. This probably explains the intermittency of evacuation reported by a number of observers (1) and the three types of response of the presumably normal human gall bladder as reported by Boyden (7). In fact, Boyden postulates variations in intramural resistance to explain the three types of response.

SUMMARY AND CONCLUSIONS

The most frequent effect on the choledocho-duodenal mechanism of the preparation of cholecystokinin used was to cause an initial increase in the resistance to the flow of fluid from the common bile duct into the duodenum; this was followed by a decrease in resistance. In most instances these changes in resistance are correlated with a periodic increase and decrease in the motility and "tone" of the duodenum. In four experiments, in which the motor response of the duodenum to the injection of the hormone was delayed, the primary effect was a decrease in resistance. This indicates that the primary increase in resistance is due entirely to the increase in the tone of the duodenum and that if the hormone has a specific action on the sphincter of Oddi, it is inhibitory rather than excitatory. These effects are obtained after cholecystectomy and are therefore independent of the gall bladder. Further evidence supporting the concept of a common duct sphincter independent of duodenal activity was noted.

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THE EFFECT OF CHANGES IN THE ENVIRONMENTAL TEMPERATURE ON THE BLOOD PRESSURE AND PULSE RATE IN NORMAL MEN

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This report presents experimental data showing the effect of changes in the environmental temperature on the blood pressures and pulse rates of normal men.

METHOD. The experimental procedure, which has been described in detail (1), is here given briefly. Sixteen normal, healthy men, who were the subjects for this experiment, came in pairs to the experimental room on two separate occasions. All experiments began about one hour after the noonday meal. On both days the subjects lay quietly, completely unclothed, for a $3\frac{1}{2}$ -hour period of observation. After the first half-hour and at every half-hour interval thereafter (a total of seven periods), the following readings were taken: room temperature, room relative humidity, rectal and oral temperatures, systolic and diastolic blood pressures, and pulse rate. The blood pressures were measured by the auscultatory method with a mercury manometer type of sphygmomanometer. The diastolic pressure was recorded at the change from the loud to the soft (fourth to fifth) sound. The radial pulse rate was counted for a full minute.

The experimental chamber was a cylindrical, brick-lined, windowless room measuring 13 feet in diameter by 8 feet in height. By the regulation of eight electric heaters, the room temperature was controlled. Fresh air was adequate and air currents were reduced so that they were imperceptible to nude subjects. Relative humidity was measured by means of a sling psychrometer.

On the first day, called the *constant heat day*, the room temperature was maintained between 23.1°C . (73.6°F .) and 23.9°C . (75°F .) for the entire $3\frac{1}{2}$ -hour period of observation. The mean relative humidity varied from 44.5 per cent to 42.3 per cent. On the second day, called the *varied heat day*, the room temperature was raised after the first series of observations were taken. The means of the room temperature for the seven successive half-hour periods were as follows: 23.6°C . (74.5°F .), 27.7° (81.8°), 30.0° (86.0°), 31.6° (88.8°), 32.6° (90.6°), 33.2° (91.7°), and 33.2°C . (91.7°F .).

Thus there was an increase of 9.6°C . (17.2°F .). The mean relative humidity percentages for the seven successive periods were as follows: 43.1 per cent, 33.3 per cent, 32.9 per cent, 31.5 per cent, 31.9 per cent, 31.3 per cent, 30.9 per cent. The changes in the relative humidity were dependent on the changes in the room temperature.

RESULTS AND COMMENT. In the first part of the accompanying table are given the mean pulse rates per minute for the seven successive periods for both the constant and varied heat days, with the differences between them. Analysis showed that the mean drop of 9.9 beats in the pulse rate per minute from 72.6 to 62.7, when the room temperature was kept constant, was statistically significant. On the varied heat day a different type of response was seen. With the greatest increase in environmental temperature during the first hour of 6.4°C . (11.5°F .) there was a corresponding increase in the mean pulse rate of 2.6. However, from this point on there was a gradual fall of 5.8 beats per minute. The drop from the third to the seventh period was statistically significant, but the change for the entire observation period was not. The differences of the means obtained on the constant and varied heat days show that as a result of the difference in the experimental conditions the mean pulse rate had increased at the end of an hour 7.4 beats per minute. This rate, however, gradually decreased to 5.8 beats per minute at the end of the $3\frac{1}{2}$ -hour period of observation.

These findings imply that some inherent mechanism was responsible for the early increase in the pulse rate in the adjustment of the body to the new environmental situation. Figures have been given by Bazett (2) and Vernon (3) showing that the pulse rate increased at a constant rate in relation to elevation of the body temperature. The influence of the body temperature does not seem to be the cause of the *early* increase in pulse rate here. Both the mean rectal and oral temperatures of these subjects showed a fall during the first hour of 0.21°C . (0.37°F .) and 0.07°C . (0.12°F .) respectively (1).

In the second part of the accompanying table are given the means of the systolic blood pressure (mm. Hg.) for the seven successive periods for both the constant and varied heat days, with the differences between them. Although there was some slight fluctuation from period to period, there were no significant changes or differences between them. Conflicting reports have been given. Sayers and Harrington (4) on exposing their subjects to saturated air at 33.1°C . (91.5°F .) for one hour noted a lowering of the systolic blood pressure while McConnell and Yagloglou (5) reported a rise on exposure to warm air. The data reported here show that neither the stimulus of an increase of 9.6°C . (17.2°F .) in the air temperature nor prolonged relaxation in the reclining position caused any significant change in the level of the mean systolic blood pressure.

In the third part of the accompanying table are given the means of the diastolic blood pressure (mm. Hg.) for the seven successive periods for both the constant and varied heat days, with the differences between them. On the constant heat day the mean diastolic blood pressure increased from 68.4 to 77.1 mm. Hg. during the 3½-hour observation period. This increase of 8.7 mm. Hg. was statistically significant. This increase in diastolic pressure as well as the decrease in the pulse rate on the constant heat day may be due not only to the constant environmental situation but also to the prolonged relaxation of the subjects maintained throughout the entire experimental period.

TABLE 1

The mean values obtained on both experimental days and the differences between them for the pulse rate, the systolic blood pressure, and the diastolic blood pressure

	SUCCESSIVE HALF-HOUR PERIODS								$p\ddagger$
	1	2	3	4	5	6	7	Δ^*	
Pulse rate per minute:									
Constant heat day.....	72.6	68.8	66.9	64.7	63.4	62.4	62.7	-9.9	$p < .01$
Varied heat day.....	71.7	73.3	74.3	71.7	69.5	68.4	68.5	-3.2	.1 < $p < .2$
Difference.....	-0.9	+4.5	+7.4	+6.4	+6.1	+6.0	+5.8	+6.7	$p < .01$
Systolic blood pressure, mm. Hg.:									
Constant heat day.....	109.8	110.0	109.5	109.9	109.9	109.8	110.5	+0.7	.6 < $p < .7$
Varied heat day.....	108.9	107.3	105.6	106.8	105.5	106.0	107.5	-1.4	.4 < $p < .5$
Difference.....	-0.9	-2.7	-3.9	-3.1	-4.4	-3.8	-3.0	-2.1	.1 < $p < .2$
Diastolic blood pressure, mm. Hg.:									
Constant heat day.....	68.4	68.9	71.6	75.3	73.6	74.3	77.1	+8.7	$p < .01$
Varied heat day.....	67.1	67.0	66.0	66.9	67.3	68.5	70.1	+3.0	$p < .01$
Difference.....	-1.3	-1.9	-5.6	-8.4	-6.3	-5.8	-7.0	-5.7	$p < .01$

* Difference between first and seventh periods.

† p = probability that the difference is due to "chance."

On the varied heat day, the mean diastolic blood pressure during the first hour did not change significantly. After the end of the first hour the mean diastolic blood pressure increased from 66.0 to 70.1 mm. Hg. at the end of the observation period. Although the increase from the first to the last period was only 3.0 mm. Hg., yet it was statistically significant. The differences of the mean diastolic blood pressures for the two experimental days reached 7.0 mm. Hg. at the end of the observation period. This difference was statistically significant. This means that the warm air caused a significant lowering in the diastolic blood pressure. Simi-

lar results have been obtained by McConnell and Yagloglou (5) and Adolph (6).

From the differences in reaction obtained between the two experimental days, an increase in the environmental temperature would seem to have affected the circulatory system in the following manner. Dilatation of the peripheral vascular bed occurs as a result of warm air stimulation. More blood is thus brought to the surface for adequate heat dissipation. The lowering of the diastolic blood pressure and the increase in pulse rate reflect the mechanisms involved to effect this change. Since the mean systolic blood pressure remained constant, the pulse pressure increased. This implies that the cardiac output became greater. Under heat stimulation through wider temperature ranges Grollman (7) has shown by direct experimentation that the cardiac output does increase. Thus to heat stimulation there seems to be a physiologic response by an increase in both cardiac rate and output.¹

SUMMARY

In a study of the effect of an increase in the environmental temperature on the systolic and diastolic blood pressures and pulse rates of 16 normal men, it was found that:

1. When the room temperature was maintained between 23.1° (73.6°) and 23.9°C. (75.0°F.) for a 3½-hour period of observation, the mean pulse rate decreased significantly—9.9 beats per minute, the mean systolic blood pressure showed no change, and the mean diastolic blood pressure increased significantly—8.7 mm. Hg.

2. When the room temperature was increased from 23.6° (74.5°) to 33.2°C. (91.7°F.) during the 3½-hour period, the mean pulse rate increased 2.6 beats per minute during the first hour, then decreased significantly—5.8 beats per minute, the mean systolic blood pressure showed no change, and the mean diastolic blood pressure increased significantly—3.0 mm. Hg.

3. A study of the differences of the two experimental days indicated that as a result of an increase of 9.6°C. (17.2°F.) in the air temperature the mean pulse rate increased significantly, the mean systolic blood pressure showed no change, and the mean diastolic blood pressure decreased significantly.

¹ Twenty-six male schizophrenic patients without discernible organic disease were subjected to the same experimental procedure. The mean general levels of blood pressure and pulse rate were slightly different from those obtained for the normal subjects. There were, however, no significant differences between these general levels for the two groups of subjects. The changes which have been described for the normal group occurred similarly and approximately to the same extent in the schizophrenic group.

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THE ACID-BASE CHANGES IN THE SERUM OF THE DOG ASSOCIATED WITH THE HYPERTHERMIA OF DINITROPHENOL ADMINISTRATION¹

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It was shown some time ago by the experiments of Collip and Backus (1920), Grant and Goldman (1920) and Davies, Haldane and Kennaway (1920-21) that voluntary hyperventilation may lead to a marked increase in blood pH and lowering of the bicarbonate concentration. During acute clinical fevers Koehler (1923) found that there was a reduction of the plasma bicarbonate concentration which was usually attended by an elevation of pH. Kast, Myers and Schmitz (1924) also observed the plasma pH to be increased above the normal level in febrile patients and Peters, Bulger, Eisenman and Lee (1926a) noted a tendency toward a lowering of the CO₂ content. Similar disturbances of the acid-base balance of the blood have been observed after the application of hot baths (Koehler, 1923), (Landis et al., 1926), on exposure to dry hot air (Cajori et al., 1923) (Danielson and Stecher, 1935) and in hyperthermia induced by a high frequency electric current (Bischoff et al., 1929-30). In all of these conditions there is an increased ventilation and hence it may be assumed that the acid-base disturbance is chiefly one of a primary CO₂ deficit.

It has been observed (Magne et al., 1932) (Cutting and Tainter, 1932) that the augmented metabolism and the increase in body temperature following the administration of 1-2-4 dinitrophenol is accompanied by a marked respiratory stimulation. Consequently it appeared of interest to see whether this drug produces a disturbance of the acid-base balance of the blood which is similar to that observed in conditions of hyperventilation. A further impetus for such a study arises from the fact that 1-2-4 dinitrophenol is extensively employed for clinical use (Cutting, Mehrrens and Tainter, 1933).

METHODS EMPLOYED. Female dogs weighing over 10 kilos were employed. They were kept on a constant diet during the entire experimental

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period. In some experiments the dinitrophenol (sodium salt of 1-2-4 dinitrophenol) was administered orally in capsules. The daily oral dose was divided into two parts, one given in the morning and the other late in the afternoon. In other experiments an aqueous solution of the sodium salt of the drug was injected subcutaneously.

The blood for the acid-base balance studies was drawn from the jugular vein under oil with as little stasis as possible. It was immediately transferred under oil into a special centrifuge tube. After the blood had thoroughly clotted, the clot was carefully loosened from the wall of the tube, and the sample centrifuged. The preservation, handling and analysis of the serum so obtained was done according to the procedures previously outlined (Muntwyler et al., 1931). In the experiments dealing with the oral administration of the drug, the blood, unless otherwise stated, was drawn in the morning before the drug or food had been given. The serum pH values were obtained with the aid of the hydrogen electrode.

RESULTS. The changes in the acid-base balance of the serum have been observed following the oral administration of 1-2-4 dinitrophenol (table 1) and following non-fatal and fatal subcutaneous injections of the drug (table 2). Five experiments were performed in which the non-fatal doses of dinitrophenol were given orally for periods as long as fourteen days and two each in which non-fatal and fatal subcutaneous injections of the drug were made. In the interest of brevity the results of only three of the nine experiments are given in tabular form.

From the data given in table 1 it will be noted that when the drug was given by mouth there was a reciprocal change in the serum chloride and bicarbonate concentrations. Thus, during the period, May 7 to May 21, the serum bicarbonate showed a decrease of 4.8 m. Eq. and the serum chloride, an increase of 3.9 m. Eq. The total measured acid value was slightly lowered. This was due in part to a decrease in the serum protein concentration. The serum total base concentration tended to be raised as the administration of the drug was continued and hence the undetermined acid value was increased. The serum pH, with some slight fluctuation, remained within normal limits. On discontinuing the drug the serum chloride and bicarbonate concentrations changed in opposite directions, that is, serum bicarbonate increased and the chloride decreased.

The blood samples in the present experiments were generally taken in the morning about twelve hours following the last administration of the drug, that is, at a time when the respiration was essentially normal. Since there is a marked increase in the rate of respiration in animals receiving the drug, one might anticipate an elevated serum pH value during the period of increased ventilation. With this in mind the blood was drawn, in a few instances, when the rate of respiration was markedly increased. When this was done no elevation of pH was observed. As a matter of

fact the pH of the blood taken two or three hours following the oral administration of the drug was found to be slightly lower than that of the blood taken before the drug was given and was accompanied by a further decrease of the bicarbonate concentration.

In general the results of the changes of the acid-base balance shown in table 1 were similar (with one exception) to those obtained in the other experiments where the drug was given orally. In some experiments where larger oral doses of the drug were given the serum chloride and bicarbonate changes were accompanied by a tendency toward a lowering of the serum total base concentration. When this occurred there was also a tendency

TABLE 1
Acid-base balance of serum following oral administration of (1-2-4) sodium dinitrophenol*

DATE	SERUM									WEIGHT OF DOG	REMARKS
	vHe	PCO ₂	HCO ₃	Cl	BPO ₄	BPr	Total determined acid	Total base	T.B.-T.A.		
1934		mm.	mEq.	mEq.	mEq.	mEq.	mEq.	mEq.	mEq.	kgm.	
May 7	7.43	38	24.5	108.9	3.2	16.0	152.6	154.4	1.8	18.0	Control, 16 mgm. sodium D.N.P. per kgm. per day
May 9	7.43	36	23.0	111.0	3.1	16.3	153.4	157.0	3.6	17.4	
May 11	7.44	32	21.1	112.7	2.7	15.9	152.4	154.5	2.1	17.5	
May 15	7.45	33	22.0	112.2	2.4	15.0	151.6	152.0	0.4	16.9	
May 17	7.40	33	19.6	113.5	2.6	14.6	150.3	157.0	6.7	16.6	Administration changed to 20 mgm. sodium D.N.P. per day
May 19	7.42	33	20.6	113.2	2.3	14.9	151.0	162.2	11.2	16.2	
May 21	7.41	32	19.7	112.8	2.6	14.8	149.9	159.0	9.1	15.9	Administration of drug discontinued May 21
May 23	7.42	37	23.4	110.8	2.1	14.5	150.8	160.2	9.4	16.4	

* The drug given twice daily. Average temperature increase in the afternoon 0.8°C. and in the morning 0.5°C.

toward a lowering of the pH from the control level. In the one experiment, which was an exception, there was a tendency for both the serum chloride and bicarbonate concentrations to decrease. Thus in this single experiment over a period of ten days during which the animal received 15 or 20 mgm. of the sodium salt of the drug daily, the serum bicarbonate concentration was found to have decreased 4.8 m. Eq. and the chloride concentration 5.5 m. Eq. At the same time the serum total base concentration had decreased 8.2 m. Eq. so that the undetermined acid value increased only slightly. The serum pH remained well within normal limits. The return of the serum chloride and bicarbonate concentrations toward

normal was slower in this experiment than in the others. It should be mentioned that the dog employed in this experiment ate only a part of its food during the period that the drug was given and this may have been a potent factor in producing the results obtained.

The results of the changes of the acid-base balance of the serum which occurred following the subcutaneous injection of the drug are shown in table 2. The findings here differ from those usually obtained when the drug was given orally in that both the serum chloride and bicarbonate concentrations tended to be lowered. It is observed that with a non-fatal

TABLE 2
Acid-base balance of serum following subcutaneous injection of (1- β -4) sodium dinitrophenol

TIME AFTER INJECT- TION	SERUM								BLOOD				REMARKS
	pHe	P _{CO₂}	HCO ₃	Cl	BPO ₄	BPr	Total measured acid	Total base	T.B.-TA.	O ₂ capacity	O ₂ content	RECTAL TEMP.	
hours	mm.	mEq.	mEq.	mEq.	mEq.	mEq.	mEq.	mEq.	mEq.	mM.	mM.	°C.	
Control	7.45	37	25.1	110.3	2.7	15.2	153.3	163.0	9.7	7.9	5.7	38.0	
1	7.46	34	23.6	105.5	2.4	15.5	147.0	156.0	9.0	9.0	6.1	39.5	Dog 2. Wt. 18.3 kgm. 17 mgm. sodium D.N.P. per kgm.
1½	7.49	29	21.0	104.0	2.1	16.2	143.3	156.2	12.9	9.0	6.7	40.3	
3½	7.44	28	18.2	105.7	2.3	15.7	141.9	156.2	14.3	8.1	6.1	40.5	
6	7.38	30	17.3	105.6	3.4	15.7	142.0	157.0	15.0	8.1	4.8	41.1	
24	7.40	33	19.8	106.7	2.6	16.1	145.2	157.8	12.6	8.1	6.1	38.4	
Control	7.48	34	24.0	107.0	2.4	16.5	149.9	149.2	-0.7	7.8	6.2	38.6	Dog 4. Wt. 20 kgm. 20 mgm. sodium D.N.P. per kgm.
1½	7.47	32	23.3	103.8	2.0	15.9	144.0	148.2	4.2	7.8	4.3	40.8	
3½	7.47	24	16.9	104.0	2.0	17.7	140.6	149.4	8.8	7.9	4.1	42.0	Dog died 3 hours and 50 minutes after injection of the drug

injection of the drug (dog 2) the decrease in serum chloride and bicarbonate concentrations was accompanied by a lowering of the serum total base concentration. This was, however, not as great as the decrease in the total measured acid value so that the undetermined acid concentration became increased. Thus, six hours following the injection of the drug the serum bicarbonate had decreased 7.8 m. Eq., the chloride 4.7 m. Eq. and the total base 6.0 m. Eq. At the same time the undetermined acid value had increased 5.3 m. Eq. It is observed that the serum pH increased during the first hour and one-half from a value of 7.45 to 7.49 and then decreased as the serum bicarbonate concentration was lowered. In another

non-fatal subcutaneous injection experiment similar electrolyte changes were observed, although the pH did not show this initial increase.

The changes of the acid-base balance observed following the fatal injection of the drug differed from those of the non-fatal injection experiments principally in that the serum total base concentration either did not change or increased slightly. Hence the increase of the undetermined acid value was somewhat greater. Further, there was definite evidence of blood concentration as death approached.

DISCUSSION OF RESULTS. It has been shown that the administration of dinitrophenol, presumably by accelerating tissue metabolism, provokes a marked increase in oxygen consumption (Magne, Mayer and Plantefol, 1932) (Cutting and Tainter, 1932). In considering the actions of dinitrophenol, Hall, Field, Sahyun, Cutting and Tainter (1933) noted that the rise in body temperature follows the increase in oxygen consumption and hence is secondary to the acceleration in metabolism. An associated phenomenon is a marked hyperventilation. The latter authors reported the changes in the plasma pH and plasma CO₂ combining power in four cats following the fatal injection of the drug. It was found that as long as the respiratory volume was maintained, there was no appreciable change in the CO₂ combining power or in pH. However, as the respiration became irregular and shallower, the pH and CO₂ combining power decreased. At death the blood pH was reported to be about 6.95. They conclude, "These results are consistent with our results on the dogs which showed that lactates did not materially increase until death approached."

It is apparent from a perusal of the data presented in the present report that variable changes of the acid-base balance of the blood in the dog may be found following the administration of dinitrophenol. It was hoped in the present study that by suitable oral doses of the drug a condition comparable to a chronic hyperventilation could be produced. The results of the changes of the acid-base balance of the serum given in table 1 we feel are those most frequently observed when this drug is given orally to dogs. It is observed in these experiments that as the serum bicarbonate decreased there was an increase of the serum chloride. This reciprocal response of the bicarbonate and chloride concentrations is similar to that observed by Peters et al. (1926b) in their experiments on involuntary hyperventilation and in their patient showing a chronic hyperventilation. Thus these authors observed that prolonged moderate involuntary hyperpnea reduced the plasma bicarbonate considerably while the increase in plasma pH was not as great as that observed in short periods of hyperventilation. Coincident with the lowering of bicarbonate there was a slight increase in plasma protein and an increase of the chloride concentration. Further their complete acid-base balance study of the plasma of a patient with a prolonged hyperventilation revealed a markedly lowered

CO_2 content and a proportionately elevated chloride concentration. The plasma pH was found to be within normal limits. With improvement of the patient's condition the CO_2 content was found to increase and the chloride to decrease. In the present experiments apparently sufficient time was given to the organism so that at the time of drawing the blood a complete compensation had been affected and hence the pH was within normal limits. When the drug was discontinued the serum bicarbonate increased toward normal while the chloride decreased.

In view of the marked acceleration of respiration which occurs in dogs receiving the drug, it was somewhat of a surprise that no elevation of pH was observed in the periods of hyperventilation. This may possibly be explained on the basis that the hyperventilation after administration of dinitrophenol is secondary to an increased CO_2 formation, whereas in voluntary hyperventilation the fall in blood CO_2 is secondary to the increased respiration. In any case it is apparent that the mechanisms of compensation were rapidly and effectively brought into play. The results of the changes of the serum acid-base balance in the experiments following the oral administration of dinitrophenol are so comparable with those which one might expect following a chronic hyperventilation, that it seems fair to conclude that the changes observed were principally the result of hyperventilation. The acid-base changes following the subcutaneous injection suggest that in addition to hyperventilation there is an incomplete oxidation of the metabolites which causes the undetermined acid concentration (organic acid) of the blood to increase.

SUMMARY

The changes of the serum electrolytes most frequently observed following the non-fatal oral administration of 1-2-4 dinitrophenol were a decrease in the bicarbonate concentration and an increase in chloride. The serum total base changes were somewhat variable and appeared to depend upon the dose of the drug given. The serum pH remained within normal limits but tended to be lowered from the control level when larger doses of the drug were given.

Following the subcutaneous injection of either a non-fatal or fatal dose of the drug both the serum bicarbonate and chloride concentrations became decreased. The serum total base concentration tended to decrease following a non-fatal injection of the drug while it tended to increase following the fatal injection. In both instances there was a definite increase in the undetermined acid concentration. The serum pH was found to remain within a comparatively narrow range. Despite this fact it is believed that hyperventilation probably played a prominent part in the changes observed in the acid-base balance.

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THE RELATIVE SIGNIFICANCE OF ELECTROLYTE CONCENTRATION AND TISSUE REACTION IN WATER METABOLISM¹

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In a consideration of the physiology of water regulation in the living organism three problems present themselves for solution. In what way is water brought to the body reservoirs, how is it held there, and how is it released? Martin Fischer (1915) concluded from in vitro experiments that acidosis caused a retention of fluid. On the other hand Schade (1925) held an opposite viewpoint, namely, that alkalosis increased the capacity of the tissues to hold fluid. Recently, Gamble and his associates (1925-1929) have emphasized their belief that the total amount of water held in the body is determined, largely, by the amount of osmotically active particles present in solution. These conflicting opinions regarding the effects of variations in the acid-base equilibrium in water metabolism have induced us to undertake this investigation.

The development in this laboratory of methods for the production of total gastric and pancreatic fistulae in dogs seemed to provide an excellent opportunity to determine the relative importance of tissue reaction and of electrolyte concentration in water retention. In 1930, one of us (L. R. D.) with Ellis described a method for producing a totally isolated stomach in the dog, leaving intact the blood supply and vagal innervation. Food passed directly from the esophagus into the duodenum and the gastric juice secreted in the isolated stomach was led to the exterior by a fistula. In spite of a liberal food and water intake the continuous loss of gastric juice was found to produce a marked dehydration, hypochloremia, alkalosis, and eventually death. Replacing the lost gastric juice by mouth or the intravenous injection of 0.9 per cent NaCl was found to be effective in relieving the dehydration and restoring the altered blood chemistry to normal.

At about the same time a new type of total pancreatic fistula was devised (L. R. Dragstedt, M. L. Montgomery, and J. C. Ellis, 1930) which per-

¹ Preliminary reports were made at the meeting of the American Physiological Society in New York, March, 1934. Aided by a grant from the Jessie Horton Koessler Fellowship Fund.

mitted the escape and collection of the total external secretion of the pancreas. Confirming other workers (Elman and McCaughan, 1927), it was found that the continuous loss of pancreatic juice produced also a severe dehydration, but with marked acidosis and eventually death. These symptoms, too, could be controlled by the parenteral administration of 0.9 per cent NaCl or Ringer's solution, while water or isotonic glucose solution were ineffective.

Both the animals losing gastric juice and those losing pancreatic juice became rapidly dehydrated as evidenced by the prompt decrease in body weight, in many instances amounting to as much as 10 per cent of the initial weight in twenty-four hours. No significant difference between the two groups with respect to the rapidity or extent of this weight loss was noted, although in the one case an extreme alkalosis and in the other an equally severe acidosis was present. It is apparent that a shift in the reaction of the body fluids with resultant alteration in the capacity of the tissue colloids to bind water is unable to prevent the loss of water which accompanies the loss of large amounts of Na and Cl ions and consequently must be of subordinate importance in determining the ability of the body as a whole to store water.

In the following experiments fistulae of the type described above were prepared in two groups of dogs of approximately the same size and general condition. Alterations in the reaction of the blood following the loss of gastric and pancreatic juice respectively were determined by measurements of the CO₂ combining power of the plasma by the method of Van Slyke and Cullen (1917). The concentration of serum sodium chloride was determined by the method of Wilson and Ball (1928). The hydrogen-ion concentration of the blood plasma was determined by the Hastings colorimetric method. Measured volumes of 0.9 per cent NaCl and 5.0 per cent glucose solutions were given by intravenous injection and the ability of the animals to retain these solutions under varying degrees of dehydration and of alkalosis and acidosis was determined by weighing at hourly intervals for 7 to 9 hours after the injection. The urinary bladder was emptied by catheter at the beginning and end of every experiment. Most of the fluid loss could be accounted for in the urine and the gastric or pancreatic juices.

RESULTS. The data are summarized in the tables. Control experiments with 5 normal dogs (nos. 1, 2, 3, 4, and 5) are illustrated in table 1. The animals were fasted for 24 hours but given water *ad libitum*. Nine hundred and eighty cubic centimeters of NaCl solution were injected intravenously in each of three animals, and at the end of six hours an average of 90 per cent of the injected fluid was excreted. The retention of 5 per cent glucose was then tested in a similar way and it was found in two experiments that all of the injected solution was excreted in three hours.

TABLE 1
Data showing the rate of water escape in the normal animal

DATE	INTRAVENOUS INJECTION	AMOUNT OF INJECTED FLUID LOST FROM THE BODY EXPRESSED IN TERMS OF PER CENT OF AMOUNT INJECTED							URINARY SECRE- TION	WEIGHT OF DOG	NUM- BER OF DOG	
		0	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.				
1934	cc.								cc.	grams		
2/20	980 of 0.9% NaCl	0	36.4	61.4	62.4	82.3	83.3	83.9	86.4	535	11,627	1
22	980 of 0.9% NaCl	0	9.8	48.1	53.2	55.5	59.8	100.0		696	13,352	2
24	980 of 0.9% NaCl	0	53.1	69.3	73.2	77.1	81.4	86.3	88.3	415	12,233	3
27	970 of 5% glucose	0	8.2	45.7	100.0					1080	16,285	4
28	960 of 5% glucose	0	49.4	49.4	100.0					1060	12,265	5

TABLE 2
Data showing the rate of water escape in the dehydrated animal

DATE	INTRAVENOUS INJECTION	AMOUNT OF INJECTED FLUID LOST FROM THE BODY EXPRESSED IN TERMS OF PER CENT OF AMOUNT INJECTED							URINARY SECRE- TION	WEIGHT OF DOG	NUM- BER OF DOG	
		0	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.				
1934	cc.								cc.	grams		
3/6	960 of 5% glucose	0	11.4	16.3	18.4	20.5	23.5	24.6	25.7	No urine	12,297	6
7	910 of 0.9% NaCl	0	8.7	19.7	25.2	30.7	34.3	36.2	38.1	140	13,070	6
21	970 of 0.9% NaCl	0	5.9	9.2	11.1	11.3	11.3	27.3	28.3	135	12,045	7

TABLE 3
*Data showing the ability of the dog with dehydration, electrolyte loss, and alkalosis to
retain water and salt solution*

Dog 8 (Total gastric fistula)

DATE	BLOOD CHEMISTRY		INTRAVENOUS INJECTION	AMOUNT OF INJECTED FLUID LOST FROM THE BODY EXPRESSED IN TERMS OF PER CENT OF AMOUNT INJECTED							SECRETIONS		
	CO ₂ capac- ity	Serum NaCl		0	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.	Urine	Digestive juice
1933	cc.	mgm.	cc.									cc.	cc.
4/25	70.1	597	970 of 5% glucose	0	19.5	49.4	50.0	52.2	56.1	78.5	79.4	650	248
26	68.5	638	980 of 5% glucose	0	42.8	43.3	47.0	69.3	70.2	272.4	73.1	449	201
27	70.6	322	990 of 5% glucose	0	35.3	63.4	46.1	76.7	77.7	80.2	80.2	610	196
28	86.5	298	975 of 5% glucose	0	44.3	47.6	49.3	74.6	75.6	76.8	77.6	612	124
29	90.0	455	990 of 5% glucose	0	41.7	43.9	45.2	46.4	46.9	50.5	71.9	600	250
5/1	109.6	450	980 of 5% glucose	0	33.1	35.0	63.2	65.1	87.0	87.5	89.0	752	205
2	127.0	393	986 of 0.9% NaCl	0	1.7	1.9	32.2	32.6	32.6	32.9	33.4	lost	271
3	122.0	425	985 of 0.9% NaCl	0	30.2	33.2	37.1	38.0	38.8	40.4	40.6	250	128
4	100.6	459	970 of 5% glucose	0	28.8	43.6	74.0	74.5	77.0	77.8	95.8	819	161
5	104.1	425	950 of 0.9% NaCl	0	0	26.6	28.8	30.3	32.8	36.5	36.5	236	127
30	95.0	398	955 of 5% glucose	0	20.4	31.6	38.4	45.2	48.0	50.7	52.3	125	108

Table 2 contains a summary of experiments performed on two dogs (nos. 6 and 7) from which water had been withheld for 12 days. In sharp contrast with the normal these dehydrated animals excreted only an average of 33.2 per cent of the injected salt solution in 7 hours and only 25.7 per cent of the injected glucose. It is perhaps significant that there was a greater retention of glucose solution than of 0.9 per cent NaCl, whereas the reverse was the case in the normal.

In table 3 are tabulated the results of eleven tests on an animal (no. 8) dehydrated and in a condition of alkalosis as a result of the loss of gastric juice. During this period the CO₂ capacity of the serum varied between 68.5 and 127.0 cc., and the NaCl concentration between 298 and 638 mgm. Eight determinations of the retention of 5 per cent glucose solution were made in this animal on different days and it was found that an average of 77.4 per cent of the amount injected was excreted in 7 hours. When a similar amount of 0.9 per cent NaCl solution was given in three tests an average of only 36.8 per cent of the amount injected was excreted in the same period.

In table 4 are tabulated the results of thirty tests on three animals (nos. 9, 10, and 11), dehydrated and in a condition of acidosis as a result of the continued loss of pancreatic juice. The CO₂ capacity of the serum in these dogs varied from normal values to as low as 26.3 cc. An average of these thirty experiments reveals that in the seven hour period 89.8 per cent of the injected 5 per cent glucose solution and 82.8 per cent of the 0.9 per cent NaCl solution were excreted.

DISCUSSION. The normal animal excreted the injected fluid more readily than was the case in any of the types of dehydration studied. As was to be expected the isotonic glucose solution was more promptly eliminated than the 0.9 per cent NaCl. The animals that had become dehydrated as a result of simple water deprivation retained both types of fluid best of all the groups. It seems reasonable to infer that these animals had lost more water than electrolyte and that as a result the osmotically active particles remaining were in a state of greater concentration than before. The marked retention of both isotonic glucose and physiological salt solutions in these cases is in harmony with the view that the electrolyte content of the organism is a major factor in determining its ability to hold water. The animals suffering from the continued loss of gastric and of pancreatic juice were in a state of more or less severe dehydration coupled with an equivalent loss of body electrolyte. Gamble and his associates have demonstrated that the digestive juices in question have the same osmotic pressure as the blood plasma. The continued withdrawal of these fluids therefore results in dehydration, but without increase in the osmotic pressure of the body fluids remaining. The fact that these animals retained isotonic glucose solution only slightly better than did normal

animals (77.4 per cent and 89.8 per cent excretion in 7 hours as against 100 per cent in the normal controls) is also in harmony with the view that the ability of the organism to retain water is dependent upon its total content of osmotically active particles. When these are reduced water is not retained, even though a severe loss of water has occurred. It is interesting that the animals in dehydration and alkalosis in every case retained water distinctly better than those with a similar dehydration

TABLE 4

*Data showing ability of dog with acidosis, electrolyte loss and dehydration to retain water and salt dilutions
(Total pancreatic fistula)*

	DATE	BLOOD CHEMIS- TRY		INTRAVENOUS INJECTION	AMOUNT OF INJECTED FLUID LOST FROM THE BODY EXPRESSED IN TERMS OF PER CENT OF AMOUNT INJECTED							SECRE- TIONS		
		CO ₂ capacity	Plasma pH		0	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.	Urine	Digestive juices
	1934	cc.		cc.	0								cc.	cc.
Dog 9	11/38	34.42	7.400	965—0.9% NaCl	0	20.2	24.3	50.8	52.9	53.2	53.6	86.0	622	120
	11/30	35.56	7.400	955—5% glucose	0	72.6	94.4	96.3	100.0				966	90
	12/1	35.86	7.437	880—0.9% NaCl	0	24.8	34.0	64.8	65.4	66.6	89.3	100.0	675	80
	12/4	31.79	7.375	910—0.9% NaCl	0	20.8	46.1	53.6	55.7	72.5	72.5	97.2	579	101
	12/5	29.64	7.360	970—5% glucose	0	67.9	68.5	100.0					975	60
Dog 10	5/12	37.4		997—0.9% NaCl	0	35.6	43.2	59.4	67.7	73.4	76.1	80.7	375	50
	5/13	33.7		972—5% glucose	0	33.7	44.4	48.2	54.2	55.2	57.9	68.6	490	60
	5/14	32.5		835—5% glucose	0	53.2	55.7	58.8	61.6	70.9	74.7	83.5	515	45
	5/18	29.1		957—0.9% NaCl	0	46.1	70.2	80.9	100.0				973	90
	5/19	29.7		988—5% glucose	0	49.8	51.7	53.1	54.2	62.3	64.0	70.4	492	60
	5/20	27.4		976—5% glucose	0	45.1	51.7	53.5	54.4	54.9	57.1	68.9	525	60
	5/21	26.3		975—0.9% NaCl	0	38.9	59.7	73.4	90.7	86.9	100.0		827	60
Dog 11	11/13	42.15	7.468	980—0.9% NaCl	0	4.5	33.6	36.0	38.0	37.0	48.2	63.0	387	60
	11/15	39.58	7.450	980—5% glucose	0	46.2	52.3	66.3	81.9	91.8	96.9	100.0	797	30
	11/16	38.38	7.510	965—0.9% NaCl	0	19.5	20.9	21.9	23.0	31.0	41.4	64.4	467	90
	11/19	36.93	7.476	965—0.9% NaCl	0	22.2	22.8	35.4	41.9	48.9	52.3	66.3	504	50
	11/23	44.45	7.545	960—5% glucose	0	45.0	57.6	63.2	77.2	79.3	83.7	89.2	618	55
	11/26			975—0.9% NaCl	0	13.5	27.6	30.7	32.0	35.5	41.5	47.7	285	50
	11/28	45.48	7.522	970—0.9% NaCl	0	13.0	15.1	15.6	29.5	Death			120	5

but with acidosis. Apparently alkalosis rather than acidosis facilitates the binding of water in the tissues. The marked retention of water given in the form of physiological salt solution by the animals dehydrated through the loss of gastric juice (only 36.8 per cent excreted in 7 hours) was not unexpected. It was surprising, however, to find that the animals losing pancreatic juice and as a result in a condition of dehydration and acidosis coupled with marked loss of fixed base (Na^+) did not retain it.

It seemed reasonable to suppose that the organism depleted of either Na or Cl ions would retain them if supplied and so increase its ability to store water.

The experiments lend support to the view of Gamble that the capacity of the organism to hold water is determined largely by the number of osmotically active particles present. The reaction of the tissues is far less important and in this case in contradiction to the views of Fischer a shift in the reaction toward the alkaline side rather than toward the acid facilitates water retention. While it seems to be true that the electrolytes (chiefly Na^+ and Cl^-) in the body tissues play this important rôle, the question then becomes of interest, what factors determine the quantity of these particles the organism retains when in a condition of normal balance. Apparently a profound disturbance is produced by the continued loss of pancreatic juice since these animals, although largely depleted of sodium ion are unable to retain it to any great extent when supplied in the form of physiological salt solution.

CONCLUSIONS

A study was made of the ability of normal and dehydrated animals to retain both 0.9 per cent NaCl and 5 per cent glucose solutions when injected intravenously. Three types of dehydration were produced. In group 1 animals were dehydrated by simple water deprivation. In group 2 animals were dehydrated and at the same time sustained severe electrolyte loss and developed marked alkalosis as a result of the continued loss of gastric juice. In group 3 animals sustained a similar water and electrolyte loss, but developed an acidosis as a result of the continued loss of pancreatic juice. The normal animals excreted the injected fluids most readily and completely. The animals with simple dehydration (group 1) retained both the glucose and salt solutions better than any of the others. On the other hand the animals that had lost both water and electrolyte (groups 2 and 3) in the proportion in which they exist in blood plasma did not retain injected water much better than normal non-dehydrated controls. The dehydrated animals sustaining electrolyte loss and in alkalosis (group 2), retained water somewhat better than similar animals in acidosis (group 3). These latter animals were extensively depleted of base, chiefly sodium ion, through the loss of pancreatic juice, but in spite of this did not retain the injected 0.9 per cent NaCl solution as well as those in group 2.

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INFLUENCE OF PARTIAL ADRENALECTOMY ON THE WORK CAPACITY OF RATS

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It has been shown by us (2) that rats anesthetized with sodium luminal and forced to continuous muscular contractions immediately after total adrenalectomy quickly lose capacity to sustain a normal rate of work and succumb within a few hours. The present study attempts to evaluate further the relation of the adrenals to homeostasis in the organism under stress by removing varying amounts of the gland from rats and comparing their subsequent capacity to survive under vegetative conditions and to resist the influence of work and anesthesia.

METHOD. The apparatus and methods employed by us in this series of experiments have been described in detail (2, 3). Rats surviving a trial dose of sodium luminal anesthesia were grouped on the basis of sex, age and weight. In the experimentals one gland and part or all of the remaining gland were removed in a single stage, aseptic operation, while the controls were subjected to a bilateral exposure of the adrenals. For complete objectivity no records were taken at this time which would identify the animal as a control or experimental, this point being established at autopsy. For the first twenty-four hours the animals stayed in an incubator at 27°C. and then were removed to living cages in a room temperature of 22° to 27°C. for a recovery period of fifteen days. The diet contained 1.3 per cent sodium chloride. Some of the totally adrenalectomized animals succumbed during the delay period. No deaths occurred among the partials or the controls and these groups were otherwise free from post-operative complications.

For twenty-five animals in ten groups, blood-sugar determinations¹ by the Folin-Wu micro-technique were made just before operation, fifteen days later immediately prior to anesthetization, and at twenty-four hour intervals during the work period. Each evaluation was an average of the readings from two separate blood samples drawn from the tail of the animal. A Pearson product-moment correlation coefficient of 0.94 from 142 double samples indicates that the method employed here is highly

¹ The biochemical analyses were made by Mr. D. L. Jacobs whose assistance was made available to us by the Civil Works Administration.

reliable and that the day to day readings indicate an actual and not merely a chance variation in level.

At the end of the recovery period the animal was anesthetized with sodium luminal. The gastrocnemius muscle was loaded with 100 grams and forced to contract three times per second by direct faradic stimulation through silver needle electrodes. Each stimulus consisted of both the make and break shock and was of optimum intensity. Six animals were worked simultaneously, the electrodes of each being connected in series with the others in the stimulating current thus allowing the same shock to stimulate each animal. The muscular contractions were registered on automatic work adders. The animal board was enclosed in a cabinet containing a water bath with temperature constant at 28°C. At eight hour intervals the experimenter recorded rate of work, administered subcutaneously

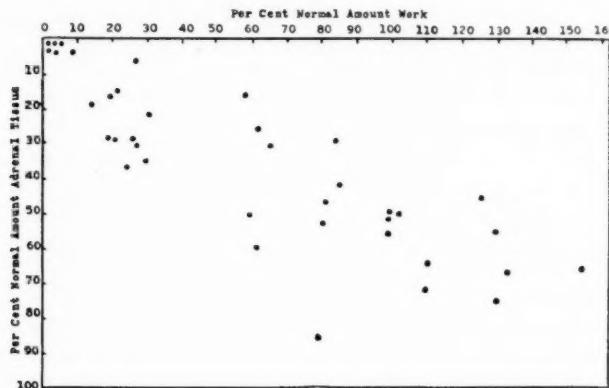


Fig. 1

0.5 cc. distilled water per 100 grams body weight, and injected appropriate amounts of anesthetic. Stimulation was continued until the death of the animal or to a maximum of 120 hours. At autopsy the adrenal tissue was removed from both the experimental and controls (both glands) and weighed to the nearest milligram.

RESULTS. For the summary of results each experimental animal was compared to the normal control of its group and its work record and amount of adrenal tissue present at autopsy expressed as "percent normal." The relationship between the work performance of the experimental animals and amount of adrenal tissue is expressed by plotting the individual data in a scatter diagram (fig. 1), while the performance of animals grouped on the basis of amount of adrenal tissue is presented as averages (table 1).

1. Of nine totally adrenalectomized rats four succumbed before the end of the fifteen day delay period and the remaining five lost weight. On the contrary, all of the partials and their normal controls survived the delay and only one failed to gain weight. The partials were otherwise completely free from overt symptoms of adrenal insufficiency. Actually, the animals with the smaller amounts of tissue tended to outgain their controls but we are not prepared to show that more than chance factors were involved.

2. When anesthetized and stimulated for contraction of the gastrocnemius muscle, the totally adrenalectomized animals quickly lost their capacity for muscular response and succumbed under these conditions of stress. The performance of these animals is similar to that reported by Gans and Miley (1) for totally adrenalectomized rats under similar experimental conditions. Among the partially adrenalectomized animals both the work accomplished and the time for survival bear a close direct rela-

TABLE I
Work averages among animals grouped on the basis of amount of adrenal tissue present at autopsy

	PER CENT NORMAL* AMOUNT OF TISSUE				
	0	0-20	20-40	40-60	60-80
Number cases.....	5.0	6.0	10.0	11.0	6.0
Work as per cent normal.....	1.05	25.0	38.2	92.0	118.0
Time hours.....	4.2	35.8	55.3	119.5	120.0

* Each experimental compared to the normal animal in its particular group.

tionship to the amount of adrenal tissue present at autopsy. Although the initial contractions compared favorably to the performance of the normals, the work curves of those animals having small amounts of tissue dropped below normal and frequently reached a state of complete "fatigue" and succumbed. It is significant that the amount of anesthetic consumed by these work-deficient animals was less than normal in every instance. Chance may account for the fact that the partials with the larger amounts of tissue tended to outwork their controls.

3. The blood-sugar level at the end of the fifteen day delay period was normal for each of the partials and was only slightly depressed for the totals even though the latter animals were in advanced states of adrenal insufficiency. After anesthetization and work there was a tendency for hypoglycemia to develop as the animal "fatigued" although in no instance was a critically low level of blood sugar observed even though the blood samples were drawn after complete "fatigue" and shortly before the death of the animal. Among the partials which resisted work and anesthetic

in a normal manner the blood-sugar level remained high until the end of the work period (120 hours) in spite of the fasting condition.

DISCUSSION. The general relationship between the amount of adrenal tissue left in the animal and the subsequent performance under stress is clearly established from the above data, but there are several sources of error which would make any quantitative expression of this an unstable value. We have not measured the amount of tissue left at operation but rather a partial gland which has undergone compensatory hypertrophy. We have made no distinction between cortical and medullary tissue in these evaluations. The dissection of a small amount of tissue from the surrounding fat and connective tissue and weighing it while losing its moisture involves some error which we believe to be within small limits. Our measurement of work involves an error which does not exceed 15 per cent as far as relative value is concerned.

The length of the delay period between operation and experimentation is an important experimental condition. Our selection of the fifteen day delay period was based upon the findings from unpublished preliminary studies by one of us (Ingle). It was shown that the removal of one gland and the partial ablation of any amount of the remaining gland was equivalent to the complete removal of both adrenals as far as immediate capacity for sustained work was concerned. It was clear from the study of performance after severe control operations that the adrenal damage was the essential experimental condition. In experimenting with various delay periods it was shown that within ten days the partially adrenalectomized animal having large amounts of tissue recovers capacity for work and that the animals having only small amounts of tissue remain permanently incapacitated for normal work although such animals show normal oestrous cycle, reproduce and lactate normally, and will survive indefinitely without presenting any overt evidence of adrenal insufficiency.

Our study of the carbohydrate level in the blood of the experimental animals was primarily for the purpose of evaluating the condition as an index for discriminating between animals having small amounts of adrenal tissue and those having larger or normal amounts. It is manifest that the blood-sugar level fails to provide such an index.

It has been generally recognized by many experimenters that adrenal insufficiency becomes more evident when the animal is subjected to stress but the wide gap between the minimum amount of tissue which will sustain life and apparent normality under optimum living conditions and the amounts of tissue necessary to maintain normality under stress has not been established in previous experiments. We recognize that our experiments do not establish any absolute values insofar as minimal amounts of tissue for maintenance of normal resistance to stress are concerned. Not only is the amount of tissue needed by the animal a function of the nature

and severity of the stress imposed but there is also an inevitable individual variability even though the experimental conditions are constant.

The present report throws no light on the question as to the relative importance of the adrenal medulla and the adrenal cortex in maintaining capacity to work under anesthesia. From results already presented (4) and from studies still in progress it seems clear that only the adrenal cortex plays the essential rôle.

SUMMARY

Totally and partially adrenalectomized rats were compared to animals that had undergone sham operations for capacity to resist the stress imposed by sodium luminal anesthesia and work of the gastrocnemius muscle as contrasted with the maintenance of life, growth, and blood-sugar level under optimum vegetative conditions. Totally adrenalectomized animals all developed overt symptoms of adrenal insufficiency during the fifteen delay period and the survivors were unable to sustain work. The partials could not be differentiated from normals on the basis of survival, weight gains, and the blood-sugar level during delay, but a strong relationship was demonstrated between the amount of adrenal tissue present at autopsy and the work performance of the anesthetized animal.

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THE SPECIFIC GRAVITY OF THE BLOOD OF NORMAL RABBITS AND CATS AND SPLENECTOMIZED RABBITS BEFORE, DURING AND AFTER EMOTIONAL EXCITEMENT

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This study was undertaken to determine the extent, duration and trend of the changes produced in the specific gravity of the blood in emotionally excited states (fear, anger and pain); the change in the specific gravity of peripheral as compared to central blood under the same conditions, and the rôle of the spleen as a contributing factor in the production of these changes. Another aspect of this study was to determine the differences which may exist between rabbits and cats with regard to specific gravity changes in states of excitement.

The literature reveals scanty data on the trend of the physical changes in the blood during emotional excitement. Barbour and Hamilton (1924) obtained indications for an increase in the specific gravity of the blood of dogs during excitement. Nice, Lindsay, and Katz (1932) reported an increase in the specific gravity of rat's blood during emotional states (anger, fear, pain).

PROCEDURE. Healthy adult rabbits and cats which were accustomed to being handled daily so as to eliminate fear as much as possible were used in these experiments. Each animal was deprived of food for 16 to 20 hours before an experiment to minimize the influence of intestinal absorption on the blood picture. The specific gravity of the blood was measured at 26°C. by a slight modification of the falling drop method of Barbour and Hamilton (1926). We used a tube 50 cm. long and 5 mm. in diameter filled with xylene-bromo-benzene solution of known specific gravity and a Sahli pipette. Blood was drawn into the Sahli pipette to the 20 mark. The pipette was inserted quickly into the xylene-bromobenzene solution and the blood let out gradually until the 10 mark was reached, and the drop released quickly.

Blood samples from the rabbits were obtained by puncturing a marginal ear vein with a needle. The ear had previously been dilated somewhat by warming with an electric bulb. The blood was obtained from the cats by making a small snip in the edge of an ear with a sharp pair of scissors.

Excitement was produced as described in our previous work. All of

the animals under this condition showed the typical signs of sympathetic stimulation.

RESULTS. Table 1 summarizes the specific gravity changes in normal and splenectomized rabbits and normal cats before, during, and after excitement. In contrast to the increase of 0.0066 in the peripheral blood in normal rabbits, the central blood samples which were obtained directly from the heart by means of an hypodermic syringe showed an increase of only 0.0034 as a result of excitement.

A series of 10 tests made on splenectomized rabbits gave 1.0472 in the quiet state and 1.0491 during excitement. In this group the basal specific gravity level after splenectomy was higher than in the normal rabbits, yet the specific gravity during excitement was approximately the same. This situation reduced the increase to 0.0018 during the excited state.

TABLE 1

Summary of specific gravity changes at 20 minute intervals before, during, and after emotional excitement

ANIMAL	TOTAL ANIMALS	FIRST NORMAL	SECOND NORMAL	EXCITED	FIRST RECOVERY	SECOND RECOVERY
Normal rabbits (P.B.).....	20	1.0421	1.0424	1.0489	1.0450	1.0414
Difference.....				+0.0066		
Normal rabbits (C.B.).....	20	1.0431		1.0465		1.0426
Difference.....				+0.0034		
Splenectomized rabbits (P.B.) ..	10	1.0472	1.0471	1.0491	1.0482	1.0473
Difference.....				+0.0018		
Normal cats (P.B.).....	10	1.0398	1.0399	1.0458	1.0419	1.0400
Difference.....				+0.0060		

P.B. = peripheral blood; and C.B. = central blood.

For comparative purposes the specific gravity of the peripheral blood in cats was measured. With these animals it averaged 1.0398 in the quiet state and 1.0458 in excitement. This increase of 0.006 is practically the same as that found in our normal rabbits.

In normal rabbits and cats there is an immediate increase in the specific gravity during excitement with a gradual recovery to the normal level following the excitatory period. Recovery took place more rapidly in rabbits than in cats. In normal cats 30 to 40 minutes after the excitatory period, the specific gravity had not quite attained its normal level; whereas in normal rabbits it had fallen appreciably below the normal level.

In normal rabbits the specific gravity had decidedly decreased in 15 to 20 minutes after the excitatory period, whereas in the splenectomized group it was at the same level as immediately following excitement. The

difference is most marked in the first 15 to 20 minutes following the excitement; thereafter recovery proceeds in both groups at approximately the same rate and in both cases the normal level is attained 30 to 40 minutes after the excitatory period.

Our splenectomized rabbits were all in good health and were used for experimental purposes at least ten weeks after the operation, so as to avoid post-operative anemia and post-operative infection.

The specific gravity of the central blood of normal rabbits shows the same general trend as that of the peripheral blood in that the increase due to excitement is immediate, and recovery is gradual. The extent of the increase however in the specific gravity was smaller in the central blood.

DISCUSSION. The above figures conform closely with 1.055 in the quiet state and 1.061 after excitement for a series of normal rats (Nice, Lindsay, and Katz, 1932).

The mechanism whereby the specific gravity of the blood is increased during excitement may be explained on the basis of three factors. First, the most important factor is the rôle of the spleen in discharging blood corpuscles during emergency states in consequence of its contractility (Binet, 1927; Barcroft 1930). This seems to account for the greatly reduced increase in the specific gravity in the splenectomized rabbits. There is evidence to indicate (Katz and Nice, 1935) that in the cat the increase in the circulating erythrocytes, leucocytes and platelets contributes to the specific gravity increase of excited blood; whereas in the rabbit only erythrocytes and platelets are to be considered, leucocytes being withdrawn from the circulation.

Second, there is the inpouring of the products of tissue metabolism into the blood stream. Katz and Nice (1934) in their studies on the chemical changes during emotional excitement reported an increase in the sugar, urea, uric acid, total and preformed creatinin, cholesterol and hemoglobin. The third factor is a slight withdrawal of plasma from active circulation during excitement in consequence of a slight shift of water into the tissues (Nice and Katz, 1934). The greater increase in the specific gravity of peripheral blood as compared to central blood indicates a shift of water through the capillaries into the tissue spaces in the periphery. These three factors combined seem to account for the specific gravity increase of blood during states of excitement.

The delay in the recovery of the specific gravity to the normal level following excitement may be explained on the basis of the work of Viale and Bruno (1927) and Menkin (1929) who have shown that during prolonged contraction of the spleen there is an engulfing and retention of the circulating corpuscles. The elimination of this engulfing mechanism in the splenectomized group may delay the return of the specific gravity to the normal level.

SUMMARY

1. The specific gravity of the blood of normal rabbits and cats showed an immediate and marked elevation during excitement.
2. Peripheral blood showed a greater specific gravity increase than central blood.
3. Splenectomy raised the basal level and diminished the specific gravity increase of excited blood.
4. Recovery in the specific gravity to the normal level is gradual and was delayed in the splenectomized group. Complete recovery was obtained within 30 to 40 minutes after the excitatory period.
5. The factors accounting for the specific gravity changes during excitement are considered.

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CORRELATED STUDIES OF CALCIUM, INORGANIC PHOSPHORUS, AND SERUM PHOSPHATASE IN NORMAL ANIMALS AND IN ANIMALS INFLUENCED BY IRRADIATED ERGOSTEROL

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The literature on hypervitaminosis D, in its relationship to calcium and phosphorus metabolism, has been recently reviewed by other investigators (1). Much of the available evidence as to the physiological significance of phosphatase suggests that this enzyme plays an important rôle in relation to bone metabolism. While the origin of serum phosphatase is uncertain, and the various factors which influence it are little understood, yet it is quite in keeping with the present knowledge of the subject to suppose that if any relationship does exist between calcium, inorganic phosphorus, and phosphatase, it might be reflected in the serum content of these constituents. The purpose of the present study was to establish, if possible, a correlation between these three serum constituents in normal animals, and to observe any change which might occur in their relationship upon the administration of irradiated ergosterol. Both subtoxic and toxic doses of irradiated ergosterol were fed, and observations continued for considerable periods of time.

EXPERIMENTAL PROCEDURE. Rabbits and dogs were employed in the experimental work, and the details of treatment will be considered under the individual experiments in which they were involved.

METHODS. All blood samples were drawn after 12 hours' fast, into oiled syringes, then transferred to oiled centrifuge tubes, and centrifuged immediately, the serum pipetted off and recentrifuged, thus minimizing the occurrence of hemolysis as well as insuring a complete removal of cellular constituents. All determinations were carried out promptly. Oxalated whole blood was used for the determination of acid soluble organic phosphorus. Determinations of inorganic phosphorus were made according to the method of Kuttner and Lichtenstein (2), applying the corrections for deviation from Beers' Law, as determined by Bodansky (3). A number of Bodansky's corrections have been verified by us, with satisfactory agreement. Calcium estimations were made by the Clark-Collip method (4), with the exception that calcium oxalate was permitted to pre-

cipitate for twenty-four hours instead of thirty minutes. Serum phosphatase activity was estimated according to Bodansky's method (5).

EXPERIMENTAL RESULTS. *Studies on rabbits.* The experimental study was divided into three parts: 1, to establish the normal level for serum calcium, inorganic phosphorus and phosphatase in adult rabbits; 2, to determine the effect of bleeding upon these values; 3, to ascertain the effect of toxic doses of irradiated ergosterol upon them.

Large adult white and chinchilla rabbits were kept on a constant ration consisting of commercial rabbit food¹ and alfalfa hay. All rabbits were

TABLE 1
The effect of bleeding upon normal serum values of adult rabbits

RABBIT NUMBER	CALCIUM* BLEEDING		INORGANIC PHOSPHORUS* BLEEDING		PHOSPHATASE† ACTIVITY BLEEDING	
	1st	2nd	1st	2nd	1st	2nd
1	13.86	16.20	4.44	2.13	4.70	2.06
2	13.37	13.60	5.62	4.92	5.23	3.70
3	13.37	15.95	6.14	3.95	4.90	4.11
4	13.74	14.90	4.83	3.62	4.77	5.88
5	12.89	13.90	3.85	3.59	3.38	1.16
6	14.10	12.29	3.75	4.78	2.79	1.55
7	13.72	13.54	4.85	4.17	4.00	1.04
8	13.98	13.78	3.37	2.89	3.38	1.29
9	12.52	12.93	5.88	3.63	4.00	2.20
10	13.01		6.94		3.88	
11	15.56		5.37		2.79	
12	13.80		5.26		4.63	
13	15.40		5.12		5.22	
14	16.10		5.16		4.26	
Average..	13.96	14.13	5.04	3.74	4.14	2.55

* Mgm. per 100 cc.

† All the data on phosphatase activity is expressed in terms of milligrams of inorganic phosphorus liberated per hour per 100 cc. of serum.

allowed a few weeks to make environmental and dietary adjustments to laboratory conditions before starting them on an experiment. The irradiated ergosterol was fed every other day by means of a stomach tube, and the control group received an equal quantity of the solvent oil (sesame oil).

Table 1 shows the normal serum values for calcium, inorganic phosphorus, and phosphatase under our experimental conditions (1st bleeding), as well as the effect of a single removal of 25 cubic centimeters of blood

¹ This food contained fats, proteins, carbohydrates, minerals, cod liver oil, etc. Rabbit Glob-ets, Albert M. Dickinson Co., Chicago, Illinois.

upon the serum level of these constituents (2nd bleeding). The second bleeding was performed from 8 to 15 days after the first. The results in table 1 show that bleeding has a definite effect upon the serum values. Table 2 shows the effect of large doses of irradiated ergosterol² upon the serum concentration of these constituents in rabbits which had not been previously bled. A comparison of the results in table 2 with those in table 1 (1st bleeding) shows that large doses of irradiated ergosterol caused a rise in serum calcium and phosphorus, and a marked decrease in serum phosphatase.

Studies on dogs. The effect of bleeding upon the serum phosphatase of rabbits led us to transfer our studies to dogs, in which the loss of blood would be relatively much less. Dogs, like rabbits, frequently show a decreased serum phosphatase on second bleeding, depending somewhat upon

TABLE 2
Serum values and weight change of adult rabbits with hypervitaminosis D*

RABBIT NUMBER	CALCIUM	INORG. P	PHOSPHATASE	DAYS ON VITAMIN D	VITAMIN D PER DAY	WEIGHT	
						Initial gms.	Final gms.
15	12.10	12.53	2.41	7	7,650 D	2140	1908
16	16.30	15.79	2.02 *	8	7,650 D	2260	1970
17	15.73	7.80	1.64	17	7,650 D	2140	1910
18	17.60	11.25	0.76	7	7,650 D	2090	1840
19	16.25	7.10	2.68	10	7,650 D	2352	2275
20	15.80	7.52	1.91	10	7,650 D	2272	2150
21	18.20	8.26	0.59	14	7,650 D	2740	2315
22	16.10	10.92	1.46	7	11,475 D	3300	2780
Average....	16.08	10.14	1.68				

* Mgm. per 100 cc.

the initial level. Probably the same factors are operative in both cases; however, the serum phosphatase of dogs, after one or two bleedings, soon reaches a reasonably constant level if the diet remains unaltered. This fact is illustrated in table 3.

The influence of sub-toxic doses of irradiated ergosterol was studied on four adult dogs (nos. 1, 2, 3, 4) which were fed a bread and corn-meal diet for a week prior to and during the experiment. After the first bleeding, dogs 1, 2 and 3 were fed irradiated ergosterol in addition to the bread and meal diet. Dog 4 served as a control. The results on dogs 2 and 3 are given in table 4. The phosphatase values on dog 4 are included in ta-

² We are indebted to the Abbott Laboratories, North Chicago, Illinois, for supplying us with the irradiated ergosterol used in these experiments.

TABLE 3

Constancy of serum phosphatase values on a bread and meal diet

DOG NUMBER	BLEEDINGS			
	1	2	3	4
2	(15)*4.20	(14) 4.29	(15) 4.69	
4	8.32	(7) 5.50	(7) 5.25	
5	(13) 3.96	(14) 3.77	(13) 4.95	
11	(14) 3.72	(15) 3.17	(14) 4.54	
12	(13) 3.25	(14) 3.77		

* The numbers in parentheses refer to the number of days since the previous bleeding.

TABLE 4

The influence of sub-toxic doses of irradiated ergosterol upon the serum calcium, inorganic phosphorus, and phosphatase of two dogs subsisting upon a bread and cooked corn-meal diet

DATE	CALCIUM*	PHOSPHORUS*	PHOS-PHATASE*	COMMENT
Dog 2, female, adult, wt. 14.5 kgm.				
2/ 3/32	11.1	3.98	5.27	Started on 1270 D/kgm. every other day
2/28	11.4	4.26	2.87	
3/ 7	11.3	3.50	5.09	Doubled dosage of ergosterol
3/14	10.7	4.90	7.55	
3/19	10.4	3.85	10.56	
3/25	10.5	2.10	8.57	
4/ 2	10.3	3.33	8.72	Dosage increased to 3200 D/kgm. daily
4/ 9	10.9	3.43	9.69	
4/16	10.7	3.89	8.63	Dosage increased to 11,800 D/kgm. every other day
4/23	13.8	4.38	6.21	Stopped irradiated ergosterol
4/30	14.6	4.08	4.75	Anorexia, lassitude

Dog 3, male, adult, wt. 17.3 kgm.

3/ 7/32	11.2	3.76	2.28	Started on 1300 D every other day
3/14	10.7	5.05	3.67	
3/19	10.4	4.81	5.44	
3/25	11.5	4.90	4.20	
4/ 2	11.4	4.78	5.36	Dosage increased to 2200 D/kgm. daily
4/ 9	11.4	3.70	8.96	
4/16	12.0	4.56	7.27	Dosage increased to 10,000 D/kgm. every other day
4/23	14.4	5.24	5.20	Irradiated ergosterol stopped
4/30	13.8	4.55	6.01	Anorexia, lassitude

ble 3. Dog 1 developed distemper after the second bleeding; the observed increase in serum phosphatase found to accompany distemper has been reported elsewhere (6). At first dogs 2 and 3 were fed insufficient irradiated ergosterol to produce an hypercalcemia, but considerable elevation of serum phosphatase resulted, apparently influenced by the vitamin D dosage (see table 4). When enough irradiated ergosterol was finally administered to cause an elevation of the serum calcium level, the serum

TABLE 5

The influence of toxic doses of irradiated ergosterol upon the serum calcium, inorganic phosphorus and phosphatase of an adult dog

DATE	PERIOD	CAL- CIUM*	PHOS- PHOR- US*	PHOS- PHO- TASE*	COMMENT
Dog 3, male, adult, wt. 17.2 kgm.					
8/13/32	1	11.5	4.42	4.41	On diet A for 8 days prior to experiment
8/19	2	11.2	4.10	3.60	
8/24	3	12.0		3.26	
8/29	4	11.9	4.50	3.07	
9/ 3	5	12.2	4.51	2.79	
9/ 8	6	12.0	4.56	2.81	
9/13	7	11.2	3.62	3.27	End of control period
Average values.....		11.7	4.30	3.71	Started on 10,000 D/kgm. every other day till 5 doses taken
9/18	8	14.3	4.65	2.43	Vomited on 21st. Some lassitude for few days, never very sick
9/23	9	16.6	3.80	1.50	
9/28	10	16.0	4.15	1.65	
10/3	11	15.1	4.12	1.65	
10/8	12	14.7	4.13	1.57	
10/14	13	14.0	5.19	1.73	
10/26	14	12.4	5.07	2.16	
Average values.....		14.7	4.44	1.81	

* Mgm./100 cc.

phosphatase simultaneously decreased in both cases. The data on the serum of these dogs suggested the possibility of an inverse relationship between serum phosphatase and serum inorganic phosphorus and/or calcium. It was decided to examine this possibility further.

Since a bread and meal diet was inadequate in many respects, subsequent experiments were carried out with a diet which was more nearly complete in nutritional requirements. For the sake of uniformity and convenience,

a commercial dog food³ with added bone meal was selected (hereafter referred to as diet A). In these experiments the dogs were bled, for the most part, at five-day intervals. Corresponding five-day samples of urine were collected and analyzed for calcium and phosphorus. Dogs 3 and 4 were placed on diet A eight days prior to the beginning of the experiment. These dogs had not been used since the previous winter (1931-1932) when the data in table 3 were obtained. After the control period (first thirty days) enough irradiated ergosterol was administered to produce definitely toxic symptoms; upon its withdrawal from the diet, recovery values were

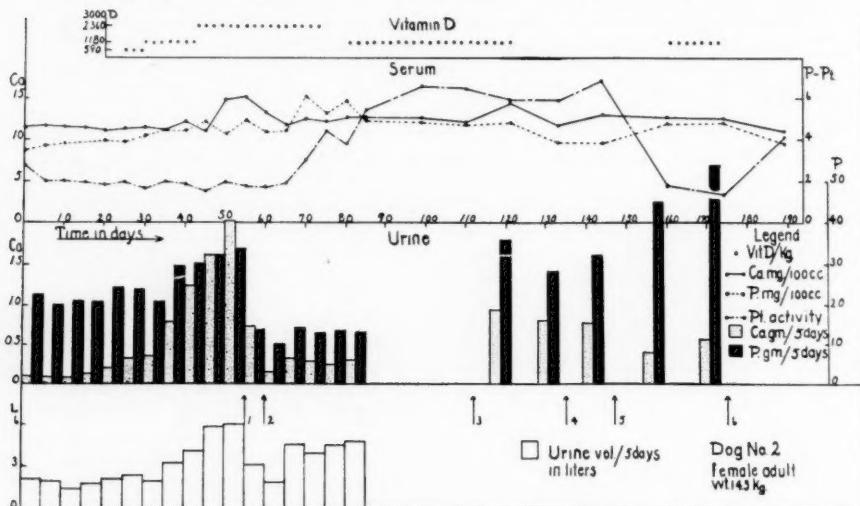


Chart 1. The influence of irradiated ergosterol upon the serum calcium, inorganic phosphorus, and phosphatase and upon the urinary excretion of calcium and phosphorus.

1, toxic manifestations. 2, substituted bread and meal for diet A. 3, added 2.5 grams each of CaCO_3 and NaH_2PO_4 to bread and meal diet.

4, replaced (3) by 2.5 grams $\text{Ca}_3(\text{PO}_4)_2$. 5, placed on a diet of beef hearts. 6, returned to diet of bread and meal.

obtained. Both dogs behaved similarly and only the results on dog 3 are presented in table 5. These results show a decrease in serum phosphatase activity without a change in inorganic phosphorus when the serum calcium level is elevated; this decrease was considered as due to the rapid onset of toxicity.

³ We are indebted to Chappel Brothers, Rockford, Illinois, for supplying us with sufficient food from a single batch of Ken-L-Ration with added bone meal for this investigation. Several cans taken at random from various cases were analysed for moisture, total ash, calcium, and phosphorus content and found to be in close agreement.

Later, five dogs (nos. 2, 3, 4, 5 and 6) were started on smaller amounts of irradiated ergosterol, gradually increasing doses being given in order to permit observations of any alteration in serum phosphatase produced by sub-toxic amounts. At the beginning of this experiment dogs 2 and 5 had been on diet A for 25 days without being bled, and the initial values for serum phosphatase were quite low and little influenced by bleeding. Dog 2, like nos. 3 and 4, had not been used for approximately six months after the data in table 3 were obtained. The results obtained with these dogs on sub-toxic doses of irradiated ergosterol show no elevation of serum phosphatase, differing in this respect from the results obtained on a bread and

TABLE 6

The influence of a dietary change upon the serum values of dogs receiving a constant dosage of irradiated ergosterol

DATE	DOG NUM- BER	CAL- CIUM*	PHOS- PHOR- US*	PHOS- PHA- TASE*	COMMENT
12/25/32	2	13.4	4.42	1.73	Receiving 2400 D/kgm. every other day.
12/30		11.9	4.50	1.97	Changed from diet A to bread and corn-meal on 12/25
1/ 4/33		12.7	6.15	3.07	
1/ 9/33		12.7	5.30	4.45	
12/20/32	3	16.9	4.64	1.52	Receiving 2000 D/kgm. every other day.
12/25/32		13.9	6.10	3.78	Changed from diet A to bread and corn-
12/30/32		15.8	6.10	2.70	meal diet on 12/20
11/30/32	4	16.0	4.50	1.42	Receiving 1100 D/kgm. every other day.
12/ 5/32		13.4	3.52	5.29	Changed from diet A to bread and meal
12/10/32		10.7	4.75	3.20	diet on 11/20
12/30/32	5	17.2	5.66	1.54	Receiving 2700 D/kgm. every other day.
1/ 4/33		13.3	4.50	1.90	Changed from diet A to bread and corn-
1/ 9/33		15.5	4.80	5.48	meal diet on 12/30
1/14/33		17.7	5.55	3.62	

* Mgm./100 cc.

corn-meal diet. The results on all 5 dogs are not included, but are illustrated by those obtained on dog 2 (chart 1).

It was found that when dogs receiving irradiated ergosterol were changed from diet A to the bread and meal diet, the phosphatase values invariably rose after a delay of a few days (see table 6 and chart 1). In case of an hypercalcemia, leaving the irradiated ergosterol dosage constant, the substitution of bread and meal for diet A caused a drop in serum calcium coincident with, or somewhat prior to, the elevation of serum phosphatase (see table 6 and chart 1). An increase in the serum inorganic phosphorus was frequently observed at this time. It was also observed, although less

marked (see table 7) that changing from diet A to a bread and corn-meal diet resulted in an increase of serum phosphatase in dogs which had not received irradiated ergosterol.

The question arose as to the explanation of the fact that diet A would not permit as high a phosphatase as was observed when the diet was shifted to bread and corn-meal. The fact that a bread and corn-meal diet is inadequate in minerals⁴ suggested itself as a possible explanation. The bread and corn-meal diet of four dogs was supplemented with salts of calcium and phosphorus, with and without irradiated ergosterol, but no conspicuous influence on the phosphatase level occurred, as is illustrated in chart 1. Another difference between the two diets is the protein content which is high in diet A and relatively low in the bread and corn-meal. In order to ascertain directly the influence of protein on serum phosphatase, the diet of various dogs was alternated from bread and meal to one of beef hearts. The decrease in phosphatase activity resulting from a high protein diet is shown in table 8 and chart 1. It was necessary to consider the possibility that sesame oil in itself might cause an increase in serum phos-

TABLE 7
Serum phosphatase activity of dogs fed diet A and bread and meal

DOG NUMBER	DIET A	BREAD AND MEAL
11	2.65	3.72
12	2.00	3.25
5	2.77	3.86

phatase, inasmuch as the irradiated ergosterol fed had been dissolved in this medium. Its influence was determined by feeding 5 cc. of sesame oil every other day for fifteen days. This was without effect, thus confirming Bodansky's opinion (9). Another dog, after receiving sesame oil for 15 days, showed an increase of serum phosphatase from an initial level of 4.69 to 6.16. However, 15 days after removal of sesame oil from the diet, the phosphatase was still high, being 5.66. This is in marked contrast to results obtained on dog 11, which, with an initial level of 4.54, showed an increase to 8.45 after receiving 1,320 D per kilogram every other day for 15 days; 15 days after withdrawal of vitamin D, it had decreased to 5.60.

A decrease in the serum phosphatase of dogs fasted for 5 days (previously fed bread and corn-meal) has been reported elsewhere (6).

In order to ascertain whether or not changes in the phosphorus compounds of the blood are associated with changes in phosphatase activity, the acid soluble organic and inorganic phosphorus of whole blood were

⁴ The bread and meal diet contained 0.072 gram calcium per 100 grams dry diet.

determined on three dogs at a number of different levels of phosphatase activity in each animal. The hematocrit reading was determined at the time of analysis on three successive bloods from two of the dogs (nos. 9 and 10), and found to be quite constant. These results are included in table 8 with other pertinent data. Although the magnitude of variation of acid soluble organic phosphorus is not very great, the highest acid soluble organic phosphorus values for whole blood are associated, for the most part, with the lowest serum phosphatase activity, and vice versa.

TABLE 8

*Serum phosphatase and acid soluble organic and inorganic phosphorus of whole blood**

DOG NUMBER	DATE	DIET	SERUM		ACID SOLUBLE PHOSPHORUS OF WHOLE BLOOD		DAYS ON DIET
			Phosphatase	Inorganic phosphorus	Organic	Inorganic	
6	4/19	B-H	3.41	5.27	15.80	4.10	14
	5/3	B-M	4.90	4.90	17.15	5.00	14
	5/17	B-M	9.26	5.08	17.41	4.34	14
	6/2	B-H	3.10	4.70	16.10	5.50	15
	6/16	B-M	7.79	4.22	17.11	5.04	14
9	4/23	B-M	14.00	4.05	14.59	3.26	18
	5/6	B-H	3.86	4.92	20.21	4.90	13
	5/20	B-M	6.81	3.82	15.89	3.66	14
	6/2	B-M	12.51	3.18	17.40	3.70	13
	6/16	B-H	3.27	3.85	21.06	4.54	14
	6/31	B-M	11.22	4.76			15
10	4/18	B-M	15.25	7.30			15
	5/3	B-H	4.77	6.75	22.08	6.92	14
	5/17	B-M	20.62	6.75	18.68	6.42	14
	6/2	B-H	4.61	6.77	21.48	6.26	15
	6/16	B-M	6.94	5.12	20.22	6.58	14
	6/31	B-M	16.47	5.46			15

* All values in mgm./100 cc.

B-H = Beef hearts

B-M = Bread and corn-meal diet

Pathological changes. The signs of toxicity observed in rabbits and dogs were, for the most part, very much the same as those enumerated by other investigators and will, therefore, not be discussed in detail (1, 13, 14, 15). The organic changes observed in rabbits fed toxic doses of irradiated ergosterol were in many instances acute in nature and resembled the changes observed on dogs reported by Taylor and co-workers (1). However, no mention has been found in the literature of the distention of the gall bladder which was observed in practically every rabbit subjected to excessive

amounts of irradiated ergosterol, whether the animal was killed or died as a result of toxicity. The gall bladders, upon aspiration, yielded four or five cubic centimeters of bile, while from each of several normal animals on our diet, only about one cubic centimeter could be obtained. The anorexia which precedes death in hypervitaminosis D may have been the cause of the distended gall bladder, although the gall bladders of two normal rabbits fasted for eight days yielded no more than one cubic centimeter of bile. This observation is difficult to reconcile with that of Russell and Chichester (7), who observed distention of the gall bladder in chickens deprived of vitamin D, although in both cases, this change may be secondary rather than due to the presence or absence of vitamin D.

DISCUSSION. Since a decreased serum phosphatase activity has been observed in fasted dogs (6) and guinea pigs (8), it is difficult to determine whether or not the hypercalcemia and/or hyperphosphatemia which we observed in hypervitaminosis D is entirely responsible for the decreased phosphatase activity. Undoubtedly the type of foodstuff metabolized has an influence upon the phosphatase activity. Our observations on rats (6), while disagreeing with those of Bodansky and Jaffe (8) on the same animal, confirm the dietary influence on serum phosphatase observed on dogs. We are in agreement with Bodansky (9) as to the influence of carbohydrates upon phosphatase activity but fail to find any consistent inverse relationship with inorganic phosphorus when diet is the sole variable. However, our experiments were of longer duration, and the apparent disagreement may be due to changes in the acid soluble organic phosphorus of whole blood. The use of a high carbohydrate or protein diet permits the experimental attainment of a high or low serum phosphatase, respectively, as may be desired.

Consideration of the various experimental procedures in which the serum calcium, phosphorus and phosphatase have been studied together, justifies the opinion that under the influence of irradiated ergosterol the serum phosphatase activity manifests an inverse relation to the serum inorganic phosphorus and/or calcium. This belief is supported by the fact that hypercalcemia and/or hyperphosphatemia following the administration of irradiated ergosterol is accompanied by a decrease in serum phosphatase activity. Also, dietary changes which reduced an existing hypercalcemia resulted in a rise in serum inorganic phosphorus and phosphatase. This rise in serum phosphatase may be permanent (chart 1) or transient (table 6) depending upon the duration of the serum calcium decrease; if the serum calcium rises again, the phosphatase will again decline. After adjustment to this dietary change, serum inorganic phosphorus and phosphatase manifest, in most instances, an inverse relationship to one another (chart 1).

Other investigators have reported results which are in accord with the

suggestion that serum phosphatase and the acid soluble phosphorus of whole blood manifest an inverse relation to one another. Kay has recently reported a decrease in the acid soluble ester phosphorus of red blood cells (10) and a high plasmaphosphatase (11) in rickets. Furthermore, Guest and Warkany (12) found that hypervitaminosis D in rabbits is accompanied by an elevation of the acid soluble organic phosphorus of red blood cells. Table 2 shows the decrease in serum phosphatase which we have observed under similar conditions.

One criticism of the phosphatase theory of calcification rests on the fact that there is practically no recognized substrate in the serum for this enzyme to act upon. However, if it can be demonstrated that serum phosphatase can make available the acid soluble organic phosphorus of whole blood, which is contained almost entirely within the red blood cells, then the objection mentioned above seems at least partially overcome. Yet, it cannot be stated that the serum phosphatase is solely concerned in activities related to bone metabolism, since two markedly different inadequate diets (bread and corn-meal, and beef hearts) result in widely divergent activities. A similar opinion has also been advanced by Bodansky (8, 9).

SUMMARY

1. Toxic doses of irradiated ergosterol increase serum calcium and/or inorganic phosphorus and decrease the serum phosphatase activity in dogs and rabbits.
2. In rabbits and dogs, bleeding causes a decrease in phosphatase activity. In dogs, after the initial lowering, the phosphatase remains rather constant.
3. A beef heart (high protein) diet induces a low phosphatase activity.
4. A bread and meal (high carbohydrate) diet causes a high phosphatase activity.
5. Sub-toxic doses of irradiated ergosterol may further increase the level of serum phosphatase activity in dogs fed a bread and meal diet.
6. The serum phosphatase of dogs fed irradiated ergosterol manifests in most cases an inverse relationship to the serum calcium and/or inorganic phosphorus.
7. Data have been submitted which suggest that an inverse relationship exists between the activity of serum phosphatase and the acid soluble organic phosphorus of whole blood.

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BASAL METABOLISM AND IODINE EXCRETION DURING PREGNANCY

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The fact that pregnancy is accompanied by a progressive increase in the basal oxygen consumption has been established by the work of the earlier investigators, particularly Magnus-Levy (1904), Zuntz (1910), Carpenter and Murlin (1911), and Root and Root (1923). Other investigators who have reported similar findings are Hasselbalch (1912), Baer (1921), Cornell (1923) and Plass and Yoakum (1929). Sandiford and her associates (1924, 1931) have concluded as the result of a study made on one subject through two successive pregnancies that the increasing heat production of the mother is proportional to the increasing surface area of the fetus and that there is no change during pregnancy in the basal energy requirement of a unit mass of the mother's tissue. This is in essential agreement with the conclusions reached by Carpenter and Murlin in 1911. Rowe (1932) opposes this view. From the analysis of a large amount of data he concludes that the excess heat production of pregnancy is the result of an unknown mechanism which involves other factors than those of fetal growth.

There is a considerable amount of evidence tending to support the view that the thyroid is involved in the metabolic reaction of the normal female to pregnancy. Anselmino and Holman (1930a, b and 1931a, b) interpret results obtained from the injection of rats with serum from pregnant women as indicating an increased activity of the thyroid gland during pregnancy. Their findings were confirmed by Soule (1932). Stewart and Menne (1933) showed that the metabolic rate of pregnant rabbits that were given Lugol's solution increased less than did that of control animals. Davis (1926, 1932) from his study of the metabolism of pregnant women who were given iodine as a prophylactic measure concluded that if normal women were given an adequate supply of iodine, their metabolic rates would remain within normal limits during pregnancy. Maurer and Diez (1926) and Scheringer (1920) have shown that the blood iodine begins to increase about the fourth month of pregnancy and reaches its highest point in the eighth month. Scheringer (1931) investigated the urinary

excretion of iodine during pregnancy and found it to be within normal limits. However from the results he obtained in a study of iodine excretion on an iodine deficient diet he concluded that during pregnancy the body was rich in iodine.

PLAN OF RESEARCH. The experiments reported here were undertaken with the following objectives in view: 1, to study the factors affecting the increase in metabolism that occurs during pregnancy; 2, to investigate urinary iodine excretion during pregnancy, and 3, to determine the effects of advancing pregnancy on vital capacity and tidal air.

Subjects. Eight subjects were investigated. Seven of these were young primiparae ranging in age from fifteen to twenty years who were inmates of a home for unmarried mothers and therefore had uniform dietary and sleeping habits. The meals were planned by a competent dietitian, and the food, although simple, was adequate. Iodized salt was used exclusively while five of the subjects were being investigated, but the last two cases were studied after the use of iodized salt had been discontinued. The eighth subject was a woman forty-four years old in her fourth pregnancy. She was living in her own home and came to the University Hospital at intervals for study. Consequently the data on this case are less complete than those on the other subjects and have been discussed only in connection with iodine excretion. She received no supplementary iodine during her pregnancy. All subjects were normal cases of pregnancy terminating in uncomplicated labor and healthy infants. None of them showed any symptoms of thyroid dysfunction.

Apparatus and procedure. All determinations of the basal metabolism were made by means of the closed circuit type of apparatus generally known as the Benedict-Roth. From the kymograph record of the movements of the bell the oxygen consumption, total calories per hour, respiratory rate, and average tidal air were all computed. At the end of each test the vital capacity was measured while the subject was still in a reclining position. The figures obtained in this way were slightly lower than those which would have been obtained had the subjects been standing, but since our purpose was merely to determine the changes in vital capacity that occur during pregnancy, this discrepancy in absolute value is of no importance, but should be kept in mind in comparing the figures given in this paper with those reported by other workers.

While this investigation was in progress, a series of five alcohol checks were run on the apparatus being used. The average error in the series was ± 0.53 per cent. The maximum negative error was -2.20 per cent and the greatest positive error was $+2.13$ per cent. We feel justified, therefore, in stating that the error inherent in the apparatus is not more than ± 2.0 per cent.

With four of the subjects a study was made of the iodine excreted in the

urine. Twenty-four hour samples of urine were collected by each of these subjects each week. The total quantity of iodine contained in the samples was determined by means of the Phillips and Curtis (1934) modification of the Fellenberg method. On several occasions duplicate determinations were made on consecutive days. By the same method it was found that the iodized salt used in the Home contained 246 parts of iodine per million. Using Kimball's (1928) figures on salt intake it was estimated that each of the subjects who used iodized salt consumed a little more than a milligram of iodine per day.

RESULTS AND DISCUSSION. The results of the metabolism studies on the five subjects who received iodized salt are shown graphically in figure 1. Inspection of the figure shows clearly that there was in every case an increase in the metabolic rate toward the end of pregnancy. This is true even when the metabolism is computed in terms of calories per hour per square meter of body surface, and the body surface is calculated by adding to the surface area of the mother the estimated body surface of the developing fetus, as was done by Sandiford, Wheeler and Boothby (1931). This increase in metabolism is of greater magnitude in the younger subjects. Es. and Ev., who were both fifteen years old, showed increases during the last ten weeks of their pregnancies of 27.4 and 18.1 per cent in total calories per hour. The corresponding increases in caloric production for J. and M., the two eighteen-year-old subjects, were 18.9 and 9.1 per cent, while the subject P. who was twenty years old increased in total heat production 11.7 per cent during a corresponding period. The increase in calories produced per hour per square meter of body surface for these subjects ranges from 18 to 7 per cent, the higher per cents again occurring in the younger subjects.

Another criterion by which we may judge the magnitude of the effect of pregnancy on the metabolic rate is the drop in the basal heat production that occurs after parturition. After delivery Es. and Ev. (both fifteen years old) showed a decrease in calories produced per hour per square meter of body surface, of 18.4 and 18.8 per cent respectively. For the two eighteen year old subjects the drop was 12.5 and 8.7 per cent and the twenty year old subject showed a drop of only 4.1 per cent. These figures are the more significant since all subjects lost about the same proportion of body weight during parturition.

In order to compare the magnitude of the mother's postpartum drop in metabolism with the basal caloric requirement of the newborn infant, the latter factor has been estimated by multiplying the surface areas of the infants, as determined by the Lissauer (1902) formula, by 26.7 (Benedict and Talbot 1915). In this comparison we find that the two fifteen year old subjects showed postpartum drops in basal heat production about three times the magnitude of the probable caloric requirements of their newborn

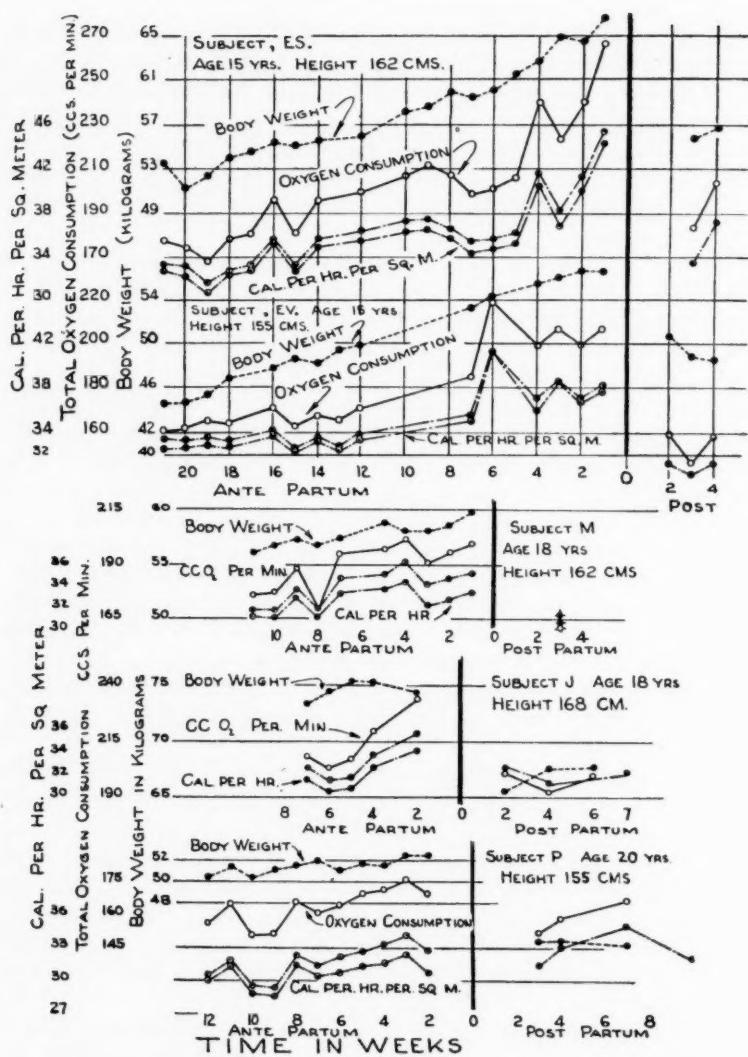


Fig. 1. Graphic representation of the changes during the latter part of pregnancy in body weight, basal oxygen consumption and calories per hour per square meter of body surface. In plotting the lower curve the surface area has been calculated by adding to the body surface of the mother the calculated surface area of the fetus according to the method of Sandiford, Wheeler and Boothby. In the upper curve the surface area has been calculated on the basis of the gross weight of the mother.

infants. With the three older subjects the postpartum drop in the mother's metabolism is in all cases less than twice the estimated caloric output of the new born infants. With the twenty year old subject the two figures are exactly the same, while with J. and M. the postpartum drop is respectively 1.52 and 1.73 times the estimated basal metabolism of the infants. The results obtained on these older girls, therefore, agree with the findings of Carpenter and Murlin (1911), while the results obtained on the two fifteen year old subjects indicate that with these younger girls there is some factor other than the caloric requirement of the fetus at work stimulating the metabolism of the mother. This is in agreement with the findings of Rowe (1932). This suggests that the disagreement between these investigators may result from the fact that they worked with subjects in different age groups. Sandiford, Wheeler and Boothby's subject was in her middle thirties, and while Carpenter and Murlin do not give the ages of their three subjects, this omission is perhaps sufficient evidence that they were all mature women. On the other hand much of Rowe's data was obtained from adolescent girls.

From an inspection of the curves of body weight and basal oxygen consumption shown in figure 1 it is apparent that with all but the subject J. there is a close correspondence between changes in body weight and variations in basal oxygen consumption. Even minor variations are in most cases parallel. This marked correlation between the changes in body weight and those in basal oxygen consumption, occurring as it does in four out of five subjects, suggests that the energy required in laying down the new tissue of the fetus and accessory structures is an important factor in producing the increased heat production of pregnancy. Our data, however, justify the conclusion that with adolescent girls there is also another stimulating factor at work which results in a more marked rise in the metabolism of such subjects than that which occurs during the pregnancy of more mature women.

The two subjects who were studied after the use of iodized salt was discontinued at the Home, were under observation only during the last month of their pregnancies and since the results obtained agree in all important particulars with those obtained on the subjects already discussed, the inclusion of detailed data on these two subjects would add nothing to this paper. One point, however, is worthy of note. The rise in the metabolism as well as the drop which occurred at parturition was of greater magnitude than that obtained with the girls of the same age who received iodized salt. One of these subjects was sixteen years old. Her metabolism dropped 18.6 per cent which is exactly the same as the drop obtained with the two fifteen year old subjects who received iodized salt. The other subject was eighteen years old and her metabolism dropped 25.1 per cent at parturition which is by far the largest drop obtained on any of our subjects.

These results are in agreement with the view that a liberal supply of iodine in the diet tends to decrease the magnitude of the rise in metabolism that occurs in pregnancy.

The results obtained in the study of urinary iodine excretion carried out on four subjects throw further light on the relation of the iodine of the diet to the metabolism of pregnancy. These results expressed in gammas

TABLE 1
Urinary iodine excretion during pregnancy

WEEK ANTE- PARTUM	SUBJECT ES.		SUBJECT P.		SUBJECT M.		SUBJECT G.	
	Cal. per sq.m. per hr.	Urinary I per 24 hrs. expressed in gammas	Cal. per sq.m. per hr.	Urinary I per 24 hrs. expressed in gammas	Cal. per sq.m. per hr.	Urinary I per 24 hrs. expressed in gammas	Cal. per sq.m. per hr.	Urinary I per 24 hrs. expressed in gammas
20	31.2	228					35.9	27*
19	33.5	158						
18	32.8	209						
17	33.3	242†						
16	35.3	146						
15	32.7	430†						58†
14								
13		116						
12	36.4	372					35.3	147†
11								
10	36.9	520						
9	37.4	152	30.9	454†				
8	38.7	408					35.6	44
7	35.2		33.3	200†				
6	35.1	175				151		
5			32.7	362	35.3	274	37.2	38†
4	41.5	147	33.4	131	36.2	910†		
3	37.8	940	34.2	1610	34.0	921		
2	41.2	240			35.5	122		
1	45.3	151			35.1	123		
POST- PARTUM								
3	33.2	250			30.7	594		

* Average of four determinations.

† Average of two determinations.

(0.001 mgm.) are shown in table 1. This table shows that the subjects who were using iodized salt (Es., P., and M.) excreted from three to ten times as much iodine as did G., who received no supplementary iodine. This may be taken as proof that iodized salt added to an ordinary diet furnishes an adequate supply of iodine during pregnancy. However there is no apparent relationship between iodine excretion and the magnitude of the increase in metabolism as pregnancy advances.

The outstanding feature of these results is the marked increase in the urinary iodine excretion which occurred in the third week antepartum in all three of the subjects that were receiving iodized salt. We can only speculate as to the meaning of this increase, but it must indicate a profound modification in iodine metabolism and possibly it accompanies a change in thyroid function. We do not know whether or not there was a corresponding rise in the iodine excretion of the subject G., for no determination of the urinary iodine was made in her case after the fifth week antepartum.

The changes that occur in vital capacity and tidal air during pregnancy were studied on seven of the subjects. In six of these (all but P.) there was a definite increase in vital capacity as pregnancy advanced, although in two of these six cases there was a drop in the last week of pregnancy. With both Es. and Ev. the increase in vital capacity that accompanied pregnancy was followed by a further increase after parturition. With four subjects the vital capacity decreased after parturition and with the remaining subject (P.) there was a gradual decrease in vital capacity during the last three months of pregnancy which was followed by a marked improvement after delivery. We feel justified, therefore, in concluding that with six out of seven subjects the increase in abdominal contents attendant on pregnancy was associated with an increase rather than a decrease in vital capacity. This is in agreement with the findings of Root and Root (1923), and does not justify the assumption often made by obstetricians that there is mechanical embarrassment in breathing during the later stages of pregnancy.

If we assume that the percentage of oxygen absorbed from the inhaled air remains constant, then the increased oxygen consumption necessary during the later stages of pregnancy must be accompanied by an increase either in the number of respirations per minute or in the amount of air inhaled at each respiration. With none of our subjects was there any noticeable change in the number of respirations per minute during either pregnancy or puerperium. With all seven of our subjects there was however a gradual increase in tidal air up to the time of parturition, which was followed by a marked decrease. We conclude, therefore, that the increased oxygen consumption of pregnancy is accomplished by an increase in the volume of tidal air.

SUMMARY

The changes that occur in basal metabolism, vital capacity, and tidal air, during the latter part of pregnancy were studied on seven subjects ranging in age from fifteen to twenty years. The urinary iodine excretion during pregnancy was studied with four subjects. The results obtained justify the following conclusions:

1. Growth of the fetus and supplementary tissues plays an important

part in producing the increased metabolism which accompanies pregnancy. With adolescent girls, however, there appears to be another factor which stimulates the metabolism and results in a greater rise with such subjects than that which occurs with more mature women.

2. The use of iodized salt furnishes an adequate supply of iodine during pregnancy and there is some evidence presented to show that this results in a decrease in the magnitude of the rise in metabolism which accompanies pregnancy. There is a marked increase in iodine excretion in the third week ante partum which suggests a possible change in thyroid function.

3. In six out of seven cases the vital capacity increased during pregnancy, and in all seven cases there was a progressive increase in tidal air during the latter part of pregnancy which was followed by a decrease after parturition.

Acknowledgments. We wish to acknowledge our indebtedness to the Board of Trustees and the Staff of the Florence Crittenten Home of Columbus. The investigation was made possible by their co-operation. We are also indebted to Dr. Andrews Rogers of the Department of Obstetrics and to Dr. George M. Curtis of the Department of Medical and Surgical Research.

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A FURTHER STUDY OF CARDIAC REFLEXES

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In a previous communication from this laboratory (Rosenblueth, 1934), some general features of reflex changes of the heart rate were reported: the influence of the frequency of afferent stimulation, and the course and duration of the subsidence of the responses. The present study deals with the influence of the adrenals, the circulatory proprioceptors, and the presence of both accelerator and decelerator afferents in mixed nerves on the cardiac reflexes obtained under the experimental conditions of the previous report.

METHOD. Cats under dial anesthesia were used. The temperature of the animals was maintained by means of a heating pad. Heart rates were recorded either by a tambour applied to the chest, or by means of a membrane manometer when changes in blood pressure were also followed.

The afferent nerves used were the left vagus and depressor, and the sciatic. Maximal rectangular electrical waves of relatively short duration (1 to 3σ) and of variable frequency were supplied by a "multivibrator."

The vagi were approached in the neck. Removal of the influence of the carotid-sinus reflexes was obtained by clamping or ligating the common carotid arteries, which, according to Heymans, Bouckaert and Regniers (1933) is an effective means of excluding these reflexes.

RESULTS. A. *The rôle of the adrenal glands.* Removal of the adrenal glands did not produce any consistent changes in either the basal heart rate or in the reflex responses studied. Ordinarily slight changes in the basal rate occurred but the variations were in either direction.

B. *The influence of the circulatory proprioceptors.* To investigate this influence, the vagi were cut, leaving as efferents only the accelerators. Cardiac reflexes were then obtained before and after clamping the carotids.

Clamping of the carotids usually induced an increase of the basal heart rate (from 230 to 243 per min., averages of 11 exp.). Two other instances in which there was a slight fall in rate are omitted.

The rise in basal rate after clamping the common carotid arteries makes it difficult to compare the curves obtained before and after this procedure. Figure 1A is representative of reflex slowing, figure 1B of reflex acceleration. It will be noticed that the same frequency of afferent stimulation produces a greater effect after than before the occlusion of the carotids,

both on the extent of the change in rate and in the form and length of the subsidence curves, especially in the early parts. A consideration of the factors involved will be found in the discussion.

Additional information as to the influence of the carotid-sinus nerves on the subsidence time may be gained by examining the interval after stimulation sufficient for subsidence to a given fraction of the elicited response. Comparison of the times of complete recovery is impracticable because of the increasing inaccuracy of the curves as they approach their asymptotes. The following table summarizes the results obtained by comparing the times for subsidence of half the elicited response in several animals when the left vagus and depressor were stimulated at various frequencies, the accelerators remaining as the only efferents.

	FREQUENCY OF STIMULATION PER SECOND						
	8	8	10	17	20	21	39
Time for $\frac{1}{2}$ recovery in {	Carotids normal	24	33	45	80	47	55
seconds	Carotids ligated	38	40	62	95	57	75
							50

An evaluation of the effects of the circulatory proprioceptors on the curves for reflex acceleration is even more difficult, since afferents yielding pure or even preponderant acceleration were only rarely encountered in this study. As will be pointed out in the succeeding section of this paper, the usual response to stimulation of the sciatic with maximal shocks is mixed, consisting of an acceleration rapidly succeeded by a slowing below the basal rate, even though the vagi are inactivated. Ligation of the carotids only rarely suppresses this rebound, and it is difficult to isolate effects which may be unequivocally attributed to proprioceptor influences. Ligation of the carotids may, however, increase the extent of the initial acceleration without materially affecting the secondary slowing. Figure 1B illustrates a somewhat unusual instance in which ligation of the carotids not only resulted in an increase of the initial acceleration, but was followed by a disappearance of the rebound as well. It should be observed, however, that the rebound was smaller than that encountered in most animals.

C. *Simultaneous stimulation of the accelerator and decelerator afferents in mixed nerves.* It is well known that most nerves, e.g., the sciatic are capable of eliciting reflexly either acceleration or deceleration of the heart. In earlier work, evaluation of the factors involved was complicated because acceleration could not be obtained reflexly in vagotomized animals unless the heart rate was reduced by constant stimulation of the peripheral ends of the vagi or by the exhibition of vagomimetic drugs. Since the introduction of dial anesthesia, however, such procedures have proved unnecessary (Rosenblueth and Freeman, 1931).

In the present experiments, shocks, maximal for all fibers, applied to the sciatic yielded varying results. In some animals pure acceleration resulted at all frequencies. Figure 2A illustrates such an instance. In others, and more commonly, slow frequencies yielded either slowing, exclusively or

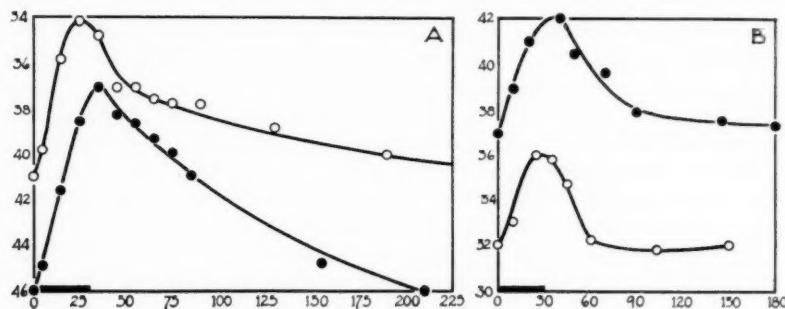


Fig. 1. Effect of removal of the influence of proprioceptors on reflex responses of heart rate. Ordinates, heart beats per 10 seconds. Abscissae, seconds. Circles, before clamping carotids; dots, after.

A. Left vagus and depressor stimulated centrally. Both vagi cut, accelerators intact. Frequency of stimulation, 10 per second.

B. As in A, but right sciatic stimulated centrally. Frequency, 17 per second.

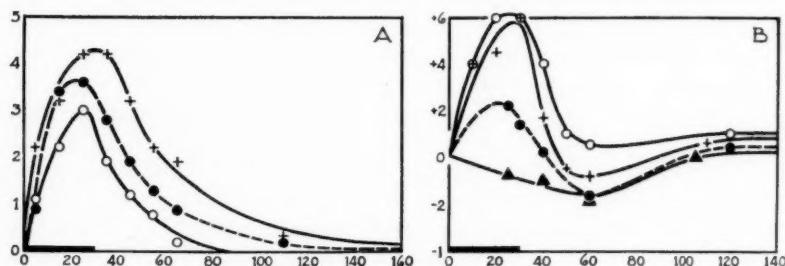


Fig. 2. Effect of varying frequency of stimulation on reflex response of heart rate. Both vagi cut, accelerators intact. Right sciatic stimulated. Ordinates, change in number of beats per 10 seconds. Abscissae, seconds.

A. Example of animal in which stimulation of right sciatic yielded pure acceleration at all frequencies. Frequencies of stimulation, 4.8 (circles), 20 (dots) and 60 (crosses) per second.

B. Example of animal in which mixed responses were obtained. Frequencies of stimulation, 11 (triangles), 29 (dots), 58 (crosses) and 82 (circles) per second.

moderate initial acceleration succeeded by slowing after stimulation ceased, while higher frequencies emphasized the acceleration and depressed or abolished the slowing. In some animals acceleration was more prominent, in others, slowing. Figure 2B illustrates a case in which both were well

marked. Attention is called to the fact that acceleration continued to increase with frequency until a frequency in the neighborhood of 80 per second was reached. This increase was consistently obtained within that range of frequency.

As a rule acceleration was easier to obtain in the first observations of an experiment. A relatively low frequency, which at first gave acceleration, might later elicit slowing, while higher frequencies which first occasioned marked acceleration were later less successful.

DISCUSSION. A. Under the conditions of these experiments, the adrenal glands were found to play no significant rôle in the responses studied. These negative results are in accord with the observations of Simeone and Rosenblueth (1934), and are probably to be attributed to the anesthesia used (dial 0.6 to 0.9 cc. per kgm.).

B. Definite statements in regard to the influence of the carotid-sinus nerves on the subsidence of reflex slowing of the heart are difficult to make since a rise in the basal heart rate was encountered upon clamping the carotids (cf. Heymans, Bouckaert and Regniers, *loc. cit.*). Strictly comparable curves will not be obtained, even though the responses be plotted as per cent of the basal rate, for the relation between central excitation and central inhibition (c.e.s. and c.i.s., Sherrington) is not lineal but such that a given degree of c.i.s. is more effective when a greater c.e.s. is present, and vice versa (see Rosenblueth, 1934). Figure 1 and table 1 would seem to indicate, however, that, at least in the early part of the curve, recovery is delayed, after the carotid-sinus reflex has been reduced to a minimum by clamping the common carotid arteries.

The influence of the carotid-sinus nerves upon reflex acceleration is also complex. Frequently in these experiments, ligation of the carotids resulted in a greater acceleration subsequent to a given stimulus, an effect which was to be expected since the carotid-sinus reflex opposes excessive increases in heart rate when the blood pressure increases. In cases in which the increase in basal rate upon clamping the carotids is marked, however, greater acceleration to the stimulus would not be expected, since the relation between the number of nerve impulses in the accelerators and the response of the heart rate is hyperbolic (Rosenblueth, 1932) so that at high rates a given stimulus results in less acceleration than at low ones. Since, therefore, all the factors involved cannot be evaluated on the same preparation, definite predictions of the relation between the amount of acceleration obtainable before and after clamping the carotids cannot be made, and, in fact, experimental results are various.

The demonstration of the rôle of the circulatory proprioceptors on the subsidence curves is especially handicapped by the fact that there is no available afferent yielding pure acceleration. In favorable instances, however, it is possible by clamping the carotids to prevent the rapid rebound in heart rate which follows reflex acceleration.

C. The fact that peripheral nerves contain afferents capable of bringing about both reflex accelerator and decelerator effects has long been known. In the present study, the use of vagotomized animals and the observation of the subsidence of the responses for relatively long periods of time make possible a more detailed analysis. As outlined above, wide variations exist in the responses; and in favorable preparations, proper manipulation of intensity and frequency of stimulation may result in changes of heart rate varying from pure slowing to pure acceleration. The most striking case is that in which intensity is kept constantly maximal, and by mere increase in frequency of stimulation pure slowing is made to give place first to acceleration succeeded by slowing, and finally to pure acceleration (fig. 2B). That the slowing is not due to the intervention of other afferents is indicated by the fact that clamping of the carotids only rarely did away with this rebound, and then only in cases of slowing which were very slight in extent; never was occlusion of the carotids successful in changing the initial slowing to acceleration. Further evidence for this view is the fact that increases in frequency progressively reduced the slowing element in all cases, just the opposite of the result to be expected if other unknown afferents were responsible.

Failure to find an explanation for the mixed responses in the activities of circulatory proprioceptors makes it necessary to look elsewhere. A tentative explanation may be made if we assume that there are differential thresholds for decelerator and accelerator afferents and differences in the rate of disappearance of e.e.s. and e.i.s. That decelerator afferents have a lower threshold than accelerator afferents is well known. The latter assumption is supported by the conclusions of Eccles and Sherrington (1931) for spinal reflexes.

The fact that, with maximal shocks, low frequencies ordinarily result in an initial decelerator reflex while high frequencies evoke an initial accelerator effect might be explained as follows. If e.e.s. disappears more rapidly than e.i.s., long intervals between successive stimuli (i.e., stimulation at low frequencies) will allow more e.e.s. than e.i.s. to disappear at the center during these intervals. Even though more e.e.s. is liberated per volley of afferent impulses the net result may be inhibitory because of the more effective summation of the more enduring e.i.s. at low frequencies. As frequency increases and the interval between the afferent volleys decreases, a point will be reached at which more e.e.s. than e.i.s. remains at the time the succeeding volley arrives, and the net effect will be one of acceleration. A similar explanation applies to the case in which an initial acceleration produced by a series of stimuli at a given frequency is succeeded by a slowing below the basal rate. At the end of stimulation there is a greater amount of e.e.s. than e.i.s. present in the center. Since the former disappears more rapidly, however, the more persistent e.i.s. becomes relatively

dominant as time passes. The heart rate, therefore, decreases more rapidly than if it were merely dependent on the rate of destruction of e.e.s., and if enough e.i.s. is present actually goes below the original basal rate.

In view of the variety of factors involved, a quantitative analysis of reflex acceleration can hardly be fruitful until a method of stimulating only accelerator afferents is devised. The same complexity no doubt exists to some degree in all peripheral nerves, similar results being readily obtained when the left vagus of the dog was used as an afferent. A few experiments, however, were carried out on the left vagus and depressor of the cat in which frequencies up to 80 per second were employed without eliciting any evidence that the accelerator afferents therein contained were numerous enough to invalidate any conclusions based upon the simplification that it acts as a pure decelerator afferent.

SUMMARY

The possible influence of the adrenal glands on the curves for reflex acceleration and slowing of the heart was investigated, and under dial anesthesia they were found to be insignificant.

The curves for reflex slowing and acceleration were compared before and after clamping the carotid arteries when the accelerators were the only afferents. It is concluded that this procedure, which reduces to a minimum the influence of the circulatory proprioceptors, tends to lengthen the subsidence time of the reflex effects and to do away with the rebound after reflex acceleration, although other factors frequently make it difficult to demonstrate the effects.

The complex responses of the heart rate to stimulation of mixed nerves are outlined. A possible explanation of these responses is found in a difference in disappearance time of e.e.s. and e.i.s.

I wish to express my appreciation to Dr. A. Rosenblueth for suggesting this problem and for his constant help in its investigation.

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PLASMA PROTEIN DETERMINATIONS IN LACTATING WOMEN

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The loss of protein in the urine by nephritis leads to a reduction in the amount of serum proteins, which is recognized as an important factor in the production of nephritic edema. Lactating women also lose large amounts of protein, often in excess of the amounts lost by the dropsical nephritis, but without development of edema. This fact raises the question as to how much loss of serum proteins, if any, occurs in the lactating woman. Being unable to find in the literature any statement of the effect of lactation on the blood protein of women, we made analyses of the blood of six normal lactating women, before and two hours after milking. The cases with the exception of one, the wet nurse at this hospital, were furnished by the Grant Hospital and The Women and Children's Hospital of Chicago.

PROCEDURE AND METHODS. Blood was taken from an antecubital vein. Care was taken to prevent, so far as possible, prolonged stasis (1). The blood was oxalated in approximately 0.2 per cent sodium oxalate concentration.

Hematoerit determinations were done in duplicate on unoxalated blood by a method similar to that employed by Capps (2). This method and control experiments on it have been described more fully by McClure, De Takats, and Hinman (3).

Nonprotein nitrogen was determined by the method of Folin and Wu (4) with the Haden (5) modification for preparing the protein-free filtrate.

Cholesterol was determined by the method of Leiboff (6).

Total solids of plasma were determined in duplicate by the method described by Myers (7). A correction was made for the sodium oxalate that had been added.

Plasma proteins, total and fractional, were determined by the method described by Hawk and Bergeim (8). This combines the method of Howe (9) for the separation of the globulins from the albumin, and the separation of the fibrinogen from the other proteins, with the method of Koch and McMeekin (10) for the nitrogen determination. The separa-

TABLE I
Data on blood of women producing mature milk

CASE	AGE	QUANTITY OF MILK OBTAINED AT ONE MILKING	WHOLE BLOOD			PLASMA						
			Hematocrit, per cent	Nonprotein nitrogen mgn. per 100 cc.	Cholesterol, mgn. per 100 cc.	Grams per 100 cc.						
						Total solids	Total proteins	Albumin	Globulin	Fibrinogen		
1	27	? Just before milking 2 hrs. after milking	{ 42.0 38.5	160.8 71.6	9.95	4.6	1.9	0.4	2.3			
			{ 43.4 39.5	175.9 037.25		4.9	1.8	0.5	2.3			
2	16	8 oz. Both breasts Just before milking 2 hrs. after milking	{ 43.5 39.0	190.9 207.8	4.8	2.75	0.25	3.0				
			{ 43.5 37.0	150.9 287.7	4.85	2.45	0.4	2.85				
3	32	4 oz. One breast Just before milking 2 hrs. after milking	{ 40.2 43.0	175.8 97.7.8	4.5	2.7	0.6	3.3				
			{ 40.8 42.0	150.9 358.25	4.55	3.0	0.7	3.7				
4	21	20 oz. Both breasts Just before milking 2 hrs. after milking	{ 43.0 45.5	200.8 187.3	4.9	2.0	0.4	2.4				
			{ 42.0 42.0	210.8 237.4	4.75	2.1	0.55	2.65				
5	25	21 oz. Both breasts Just before milking 2 hrs. after milking	{ 39.0 33.5	180.8 387.0	4.6	2.1	0.3	2.4				
			{ 39.0 32.0	185.8 567.35	4.6	2.55	0.2	2.75				
6	18	15 oz. Both breasts Just before milking 2 hrs. after milking	{ 42.3 39.0	185.8 567.75	4.55	2.7	0.5	3.2				
			{ 40.8 40.0	130.8 617.4	4.7	2.4	0.3	2.7				
Normals from literature.....			38.0 25.0	165.7.0	6.2	3.6	*		2.0			
			to to	to to	to to	to to			to			
			45.0 35.0	230.9.0	8.0	5.0			3.5			
Average for men and women.....			41.0		8.0 7.1	4.5			2.7			

* Most workers who have used the Howe method for the separation of the albumin from the globulins, have reported their figures for the globulins including the fibrinogen, hence we have quoted the range from the literature for this combination. Fibrinogen figures are given by various other methods as ranging from 0.3 to 0.6 gram per 100 cc. of plasma for the normal.

tions of the fractional proteins were not carried out in duplicate but all nitrogen determinations were checked in duplicate.

The results are given in table 1.

It will be seen that the considerable losses of protein in the milk have no recognizable effect on the blood proteins, which are in all cases in normal quantities. Evidently, then, the excretion of casein, apparently formed from the free amino acids of the blood (11), is not demonstrably at the expense of the plasma proteins, at least under conditions of adequate nourishment. Whether with inadequate protein intake the mammary glands will excrete casein at the expense of plasma proteins has not been determined, as far as we can learn.

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INFLUENCE OF THE OVARIAN HORMONES, OESTRIN AND PROGESTIN, UPON THE MENSTRUAL CYCLE OF THE MONKEY

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The reasons for undertaking the following experiments will be apparent to all who are interested in the problem of menstruation, and this report requires therefore no general introduction. Readers who wish such an orientation are referred to a recent essay on the nature of the menstrual cycle by the present author (Corner, 1933).

The animals used were eleven specimens of the common Rhesus macaque, currently called by taxonomists *Macaca mulatta*. Although this is the only primate species suitable at present for experimental study of the reproductive system, its menstrual cycle is not precisely like that of the human female. The differences, which must be taken fully into account, will be understood from the monographs of Corner (1923) and Hartman (1932). In brief, the female Rhesus monkey (as observed under laboratory conditions in North America) menstruates with fair regularity between September and April, with a modal cycle length of about 28 days; during the warmer months, May to August, irregularity and infrequency of menstruation, amounting almost to amenorrhea, is the rule. For this reason experiments involving the menstrual rhythm must be made between September and April, and when (as in the present work) the expected result is delay or suppression of the menses, the experiment must be preceded by a control period of one or two cycles in the autumn, to give assurance that the monkey is in good condition.

Even during the season of more or less regular menstruation, moreover, irregularity is greater than in healthy human females. The modal cycle length varies from animal to animal, and deviations of two or three days in either direction from the mode are very frequent. Occasionally a larger deviation, or the total omission of one menstrual period, occurs in an otherwise regular cycle, as will be seen from some of the protocols given below. In work of the kind reported here, it is therefore obviously improper to draw conclusions from any single experiment, for it is impossible to know whether a disturbance of menstrual rhythm is spontaneous or due

to the experimental treatment, and a constant outcome of repeated trials is necessary for decision.

PART I. *The effect of oestrin.* Among the few clues which we have as to the endocrine relations of the menstrual cycle, the most important is that which points to the ovarian hormone, oestrin, as in some way a factor. The evidence, which is given in full in the paper above cited (Corner, 1933), has been by some interpreted hypothetically to mean that the menstrual hemorrhage is due directly to a cyclic reduction of the oestrin level of the body, which removes a protecting influence of oestrin upon the endometrium. If this were true, it would be expected that the maintenance by artificial means of a high oestrin level would prevent the endometrial disturbance and thus abolish the menstrual flow. The following experiments were planned to test that hypothesis.

Very little has been published on the subject. Hartman (1934) cites one experiment on a Rhesus monkey in which injection of a total of 995 rat units of oestrin (Amniotin Squibb) in eight days, beginning seven days before the expected onset, did not delay menstruation. G. Van S. Smith, in discussing Hartman's report, mentioned that 9 human patients with regular cycles who received from 200 to 600 rat units daily by mouth (Schering's Progynon) showed no discernible effect upon the rhythm of menstruation, except that in two of the longer trials the flow seemed to be somewhat more scanty than usual. On the other hand Bowman and Bender (1932) reported that in four of five patients given monthly courses of oestrin (Amniotin) menstruation did not occur during the period of treatment.

In all the present experiments, with one exception, oestrin was used in the form of Progynon, a benzoic acid ester of ketohydroxy-oestrin, which has the advantage, first reported by Butenandt and Störmer (1932) and confirmed by Schoeller, Dohrn and Hohlweg (1932) that unlike ketohydroxy-oestrin itself, it gives a greatly prolonged effect from a single dose, and thus injections may be less frequent than with other forms of the drug, a great convenience when it is a matter of handling monkeys. In rats a single subcutaneous injection of one ^{1/2} mg produces vaginal cornification for a week; the monkeys were given 1 subcutaneously thrice weekly, and in every case gave evidence by the condition of their sexual skin that a continuously high oestrin level had been produced.

I am greatly indebted to the Schering Corporation, and especially to Dr. Gregory Stragnell and Dr. Erwin Schwenk, for supplying generous quantities of Progynon. The substance furnished me in the autumn of 1932 is designated Progynon benzoate in the present paper and protocols; that furnished in 1933-34 is designated Progynon-B. I am informed by Doctor Schwenk that the former compound was the benzoic acid ester of Theelin (i.e., of ketohydroxy-oestrin); while Progynon-B as issued in 1933

and thereafter is the benzoic acid ester of dihydro-Theelin (i.e. mono-benzoic ester of dihydroxy-oestrin). Each lot of the drug used was tested on rats in our own laboratory and the material was uniformly found to equal the potency indicated by the manufacturer.

The dosage was determined by the following considerations: 1, Robertson, Maddux and E. Allen (1930) induced reddening of the sexual skin in an adult castrate macaque with about 10 rat units of oestrin (Amniotin) daily during 48 days; 2, Morrell, Powers, Varley and de Frates (1930) obtained bleeding in the castrate after a six days' course of Amniotin, 12.5 rat units per day; 3, Saiki (1932) inhibited with 15 rat units of Amniotin daily the menstruation-like bleeding which follows a course of anterior pituitary extract; 4, Hisaw, Meyer and Fevold (1930) found 30 to 40 rat units of Amniotin per day for 10 days sufficient to prepare the castrate monkey's uterus for the action of progestin.

With these considerations in mind, the dosage was at first at the rate of 15 rat units daily, given by hypodermic injection every second day. When it was found in the earliest experiments that this dose did not inhibit the first menses after the start of injections, variations were tried, namely, a gradually increasing dosage, and larger doses from the start; some of the animals therefore received as much as 90 rat units daily. Since it appears that differences in the dosage did not affect the result, it is not necessary to repeat here the details, which will be found in the protocols.

Eight of the thirteen experiments (nos. 57 (1), 57 (2), 66, 70 (1), 85 (1), 85 (2), 90 (1), 90 (2)) gave similar results in that the first expected menstrual flow after the beginning of the treatment was not affected; as the injections continued the next return of the periodic bleeding was delayed, occurring from two to eight weeks later than the expected time; but these delayed menses, or at least menstruation-like bleeding, finally occurred in spite of and during the course of treatment. To these 8 experiments may be added one more (no. 64) in which events proceeded as above but the animal was killed before the return of bleeding, and another (no. 105) in which the first two menses occurred on time but the delay which followed cannot be properly taken into consideration for a reason cited in the protocol. In one (no. 106) total suppression of uterine bleeding was observed. In a single animal (no. 102) the treatment had no effect upon the menstrual rhythm.

Thinking that possibly Progynon might not have the same action in every respect as natural (ovarian) oestrin, a supply of ovarian follicle fluid from sows was extracted, and the partially purified oestrin thus prepared was administered to macaque no. 70 (2nd experiment) at the rate of 30 rat units per day, lasting long enough to show that like Progynon, it did not inhibit the first expected menses. The uncertainty which prompted this particular experiment has been lessened by the recent report of MacCor-

quodale, Thayer, and Doisy (1935) that oestrin as extracted from the follicle fluid is dihydro-Theelin (i.e., dihydroxy-oestrin).

In all, twelve experiments out of thirteen gave a consistent result with regard to the first menses.

Certain important accessory facts were discovered in the course of the experiments. First, in all animals which were put through the complete experiment, the color of the sexual skin (which, as is well known, serves as a qualitative indicator of the action of oestrin) became more intense, or if bright at the beginning remained so, as would be expected from the action of the hormone. Although the color generally fades at the onset of natural menstruation, it was not affected by the first period of bleeding which occurred during the experimental treatment. At about the time of the delayed bleeding which finally came on during the experiments, however, the color of the sexual skin faded out. Second, in those experiments which were terminated early in the season, before the approach of the usual summer oligomenorrhea, menstrual cycles were resumed after the discontinuance of the injections, usually coming about four weeks after the delayed bleeding which occurred under treatment.

Third, when the animals were explored surgically at the end of the experiment (i.e., during or just after the delayed bleeding) it was found that none of them had recent corpora lutea in the ovaries; in other words, they had not ovulated during the experiment. This observation, it must be admitted, was not fully controlled by a knowledge of the ovulation-rates of the same animals before the experiment; and yet the non-occurrence of ovulation seems significant in view of the facts that 1, the season was that in which ovulations are most common; 2, one of the animals was known to have ovulated in previous winters and two were known by direct exploration to have ovulated just before the beginning of the experiment.

For the sake of comparison with this experimental series, I have compiled from the general records of our monkey colony all the relevant observations (by surgical exploration or by autopsy) made on animals not under experiment, and of comparable age to those used in the experimental series, in which by actual ocular evidence of the presence or absence of a recent corpus luteum it was determined whether or not the animal had ovulated during that period of the seasons 1932, 1933, 1934 corresponding to the period of the experiments, namely, January, February, March. In this control series ovulation had occurred in nine out of eleven observations. Such a contrast, namely, 80 per cent ovulation in the controls, zero per cent in the experiment, can scarcely be the result of chance, and we must conclude that oestrin, in the form and dosage used, inhibits ovulation in the monkey. This observation, if applicable to the human species, has an important bearing upon the use of oestrin medication in disorders of the reproductive function.

The character of the delayed menstruation-like bleeding which took place in these experiments was investigated by biopsy of the uterus in two cases (no. 70, 90 first experiments), and was found to resemble exactly the natural periodic hemorrhage or non-ovulatory menstruation which frequently occurs in monkeys; the superficial third of the endometrium had broken down and bleeding was occurring from an endometrium of the interval type. I have refrained from applying the term "menstruation" to this delayed bleeding, and yet it cannot be distinguished by any possible means from natural menstruation of the non-ovulatory type, which it resembles in extent and duration, in its chronological relation to ensuing cycles after the termination of the experiment, and in histological characteristics.

The one animal subjected to histological examination during the phase of delay (no. 64) exhibited an endometrium in full interval development, exactly as occurs in the normal cycle with a maturing follicle; this histological stage is well known to be reproducible with oestrin in the castrated monkey.

PART II. *The effect of progestin.* The following observations are the first of their kind to be reported. The only previous work which is relevant is that of Smith and Engle (1932), which has very recently found confirmation in a few experiments by Hisaw (1935). Smith and Engle tried the effect of progestin upon experimentally induced menstruation-like bleeding of castrated monkeys, which they caused to occur by means of bilateral oophorectomy combined with abrupt cessation of a course of anterior pituitary extract and of oestrin (Theelin). Giving daily an amount of progestin equivalent to 70 grams of fresh sow corpora lutea, which I estimate would be approximately 2.5 rabbit units by our standard test (Corner and W. M. Allen, 1929) they could inhibit the bleeding for as long as 28 days. The progestin caused a typical progestational state of the endometrium. After cessation of the progestin treatment, bleeding occurred within six days.

In the following experiments the progestin used came from four separate lots prepared in our laboratory by a method based on the work of W. M. Allen and Meyer (1933). The method makes use of separation by immiscible solvents (aqueous alcohol and petroleum ether) and yields a preparation having about 12 to 20 mgm. solids to 1 rabbit unit, which in quantities such as administered in these experiments contains no detectable amount of oestrin. The potency of each batch of this progestin was established by our standard test on adult rabbits (Corner and W. M. Allen, 1929). The extract was diluted with sesame oil in proportions arranged to make 0.25 cc. contain one daily dose. Sesame oil in such quantity is well tolerated by monkeys in daily subcutaneous injections; an effect of the sesame oil itself upon the menstrual cycle is rendered unlikely by reason

of the very different effects given by the Progynon and the progestin, both of which were administered in this oil.

The plan of experiment was as follows: each animal was allowed one or more control cycles; the time of the next expected menses having been estimated as closely as possible from the history, treatment with progestin was begun about a week before the expected onset.

The result was unequivocal, as will be seen from the protocols. Six animals received daily doses of 1 rabbit unit per day. In three of these (nos. 70 (1), 102, 115) the next menstrual period expected after the beginning of treatment was definitely postponed until after cessation of treatment. In no. 70 (1) an interval of 54 days was thus produced; in no. 102 an interval of 45 days; in no. 115 an interval of 50 days. In each of these cases cessation of treatment was followed in 5 to 8 days by external bleeding of menstrual type. In each case also typical menstruation occurred again at the normal interval of approximately four weeks; and in nos. 102 and 115, which were not subjected to further experimentation, it became clear that the menstrual cycle had simply been shifted by a time interval corresponding to the delay. No. 116 was given the same dose, and the result was slight microscopic bleeding (detected by vaginal lavage) beginning about a week after the expected date of onset of menstruation, persisting about 9 days, but definite menstruation did not occur during a total interval of 47 days. Typical menstruation began 5 days after withdrawal of progestin. Here again subsequent cycles followed in approximately four-weekly intervals after the experimentally shifted menses. No. 106 (2) was also given 1 rabbit unit of progestin daily. Menses expected some time between February 19 and March 1 not having occurred by March 2, a biopsy was performed and the findings (a corpus luteum in early degeneration with a typical premenstrual endometrium) indicated that in this experiment menstruation had been delayed at least a few days beyond its expected onset. In no. 66 the cycles of the control period were too irregular to enable prediction of the next menses, but administration of 1 rabbit unit of progestin daily was accompanied by an interval without menstruation amounting to 39 days. Cessation of injections was followed by menstruation-like bleeding 7 days after the last injection, making a total interval of 46 days. Biopsy of the uterus showed that although the animal had not ovulated during the past two months, the endometrium had developed a typical premenstrual state under the influence of the progestin. The endometrium would certainly be taken, from its histological picture, to be that of natural menstruation of the first day. Menses recurred after 30 days.

From these six experiments, therefore, it seems demonstrated that the effect of progestin is to prevent natural menstrual bleeding, and that the withdrawal of progestin treatment is followed after 5 to 8 days by spon-

taneous bleeding from the endometrium, having in general the duration and appearance of typical menstruation, and occurring from an endometrium of the premenstrual type. That such bleeding may be considered equivalent to normal menstruation is further indicated by the fact that in those cases not complicated by subsequent experiment, spontaneous menstruation followed in a typically cyclic manner at four-weekly intervals.

Two animals were tried with daily doses of 0.5 rabbit unit. In no. 70 (2) menstruation seems to have been delayed with a total interval of 45 days. In no. 90 the experimental interval was 33 days, which cannot be interpreted as a definite delay, but treatment had been begun very late, in fact within two or three days of the expected onset. Evidence of progestin action was given by the fact that withdrawal of the treatment was followed in 5 days by menstruation-like bleeding, and it is possible that in future experiments 0.5 rabbit unit will prove to be a sufficient dose. A dose of 0.1 rabbit unit, given to monkey 106 (1) had no effect.

CONCLUSIONS

The first conclusion to be drawn from these experiments is that the corpus luteum hormone, progestin, has the definite property of inhibiting the menstrual flow in the monkey. Such a finding is of course entirely compatible with our general knowledge of the reproductive cycle, and also agrees with the experiments of Smith and Engle (1932) cited above, in which it was found that experimentally-induced menstruation-like bleeding could be inhibited by doses of progestin amounting to 2.5 rabbit units daily. The second conclusion is that simply maintaining the oestrin level by injections of Progynon-B or crude follicular oestrin is not sufficient to prevent the onset of natural menstruation. The subsequent delay of menstrual periods after the first, which occurred almost uniformly during continued treatment with Progynon, should probably not be taken into account in formulating hypotheses as to the endocrine relationships of the menstrual cycle. It is well known that continued administration of oestrin decreases the production of gonad-stimulating hormone by the hypophysis and it is therefore probable that during the later stages of a course of oestrin treatment deep-seated disturbances of the pituitary-ovarian balance may be produced. It will be recognized, however, that the results here presented with regard to the non-interference with menstruation in the earlier part of the course of treatment with oestrin cannot be reconciled in any simple or direct way with the oestrin-deprivation theory of menstruation. Further experimental studies will be required before a coherent explanation of the menstrual cycle can be constructed.

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Thanks are due Dr. Willard M. Allen for chemical advice in connection with the supply of progestin, to Mrs. Bessie L. Goff for assistance in the experiments, and to Mr. Frederick Kesel for unceasing care and observation of the animals.

PROTOCOLS. PART I. Experiments with oestrin (Progynon and follicle extract). *The expression "control period" signifies the entire menstrual history during the season of relatively regular menstrual cycles, and includes all intervals of a given season from October to April.*

No. 57, first experiment. Control periods:

Season 1929-30. Intervals of 24, 23, 30, 38, 32, 26 days

Season 1930-31. 27, 24, 30, 20, 35, 27, 28, 25.

Season 1931-32. 30, 29, 28, 29, 29, 28, 31, 24.

Season 1932-33. 30, followed by experiment.

Last control menses began November 28, 1932. Given Progynon benzoate December 5 to December 12 at rate of 13.3 rat units per day, December 12 to December 23 at rate of 16.3 rat units per day, December 24 to January 21 at rate of 29 rat units per day, January 22 to March 1 at rate of 30 rat units per day.

Result: Menstruation-like bleeding January 2-6; again February 23-27. Eight days after discontinuance erythrocytes in washings for a few days.

Interpretation: first menses during treatment not significantly delayed; next menses delayed about 3 weeks, but occurred during treatment.

No. 57, second experiment. Control periods: As above, with the addition of Season 1933-34, 25, 29, 30, followed by experiment.

Last control menses began November 25, 1933. Given Progynon-B December 18 to March 19 at rate of 90 rat units daily.

Result: Menstruation-like bleeding December 28-30; thereafter no bleeding until March 22-26 (beginning 3 days after cessation of treatment).

Interpretation: first menses during treatment not significantly delayed; no further menstruation during experiment, i.e., a delay of at least 8 weeks.

No. 64. Control periods: Season 1932-33. Intervals of 32, 39 days.

Last control menses began January 5, 1933. Given Progynon benzoate January 20 to March 8 at rate of 30 rat units daily.

Result: Menstruation-like bleeding January 23-27 (18 days after onset of last menses); no further bleeding. The sexual skin became highly edematous and moderately reddened. No further bleeding having occurred, the animal was killed March 9, 45 days after onset of the last menses. Histological findings are discussed in the text (p. 242).

Interpretation: first menses during treatment not delayed; no further menstruation during experiment, i.e., a delay of at least 2 weeks.

No. 66. For control periods, see protocol of same animal below, p. 247.

Last control menses began November 21, 1932. Given Progynon benzoate November 28-December 13 at rate of 13.2 rat units per day; December 14-21, 15 rat units per day; December 23-January 21, 29 rat units per day; January 21-March 6, 30 rat units per day.

Result: December 22-27 a few erythrocytes in vaginal washings; March 4-10 menstruation-like bleeding. Five days after cessation of treatment a few erythrocytes in washings.

Interpretation: apparently partial suppression of first expected menses; 4 weeks' delay of next menses. In view of previous irregularities in the cycle this experiment is not significant when taken alone, but the menstrual course during the experiment is strikingly similar to that of no. 57.

No. 70, first experiment. For control periods, see protocol of same animal below, p. 248.

Last control menses began November 27, 1932. Given Progynon benzoate December 5-13 at rate of 13.3 rat units per day; December 14-January 8, 1933, 16 rat units per day; January 9-March 9, 30 rat units per day.

Result: Menstruation-like bleeding December 26-29; again March 2-6. After discontinuance, a few erythrocytes in washings daily for a fortnight.

Interpretation: first menses during treatment not delayed; next menses delayed about four weeks but occurred during treatment.

No. 70, second experiment. Last control menses began December 19, 1933. Given follicular oestrin in sesame oil (see text, p. 240) 30 rat units daily in 2 doses of 15 rat units each January 8-24.

Result: Menstruation-like bleeding January 17-22.

Interpretation: there is no difference between Progynon ester and follicular oestrin, as concerns their effect in not stopping the first menses after beginning of injection.

No. 85, first experiment. Control periods:

Season 1931-32. Intervals of 29, 28, 27, 22, 36, 32, 23 days.

Season 1932-33. 30, followed by experiment.

Last control menses began November 21, 1932. Given Progynon benzoate December 9-13 at rate of 12 rat units per day; December 14-January 8, 1933, 16 rat units per day; January 9-March 6, 30 rat units per day.

Result: Menstruation-like bleeding December 20-24; again February 25-March 4. March 15-23 (beginning 9 days after cessation of treatment) menstruation-like bleeding.

Interpretation: first menses during treatment not delayed; next menses delayed about 5 weeks, but occurred during treatment.

No. 85, second experiment. Last control menses began November 29, 1933. Given Progynon-B December 16-March 19, 1934 at rate of 60 rat units per day.

Result: Menstruation-like bleeding, December 25-29; again February 2-3, 1934, and March 19-23. No bleeding again until 29 days after cessation of treatment.

Interpretation: first menses during treatment not delayed; next two delayed about 2 and 2.5 weeks respectively, occurring during treatment.

No. 90, first experiment. For control periods, see protocol of same animal below, p. 248.

Last control menses began November 29, 1932. Given Progynon benzoate December 9-13 at rate of 12 rat units per day; December 14-January 8, 1933, 16 rat units per day; January 9-March 2, 30 rat units per day.

Result: Menstruation-like bleeding December 19-22; again February 27-March 7.

Interpretation: first menses during treatment occurred about one week early; next delayed about 6 weeks, occurring during treatment. Owing to irregularities during previous seasons, the experiment is not significant alone, but closely resembles others of the series.

No. 90, second experiment. Last control menses began December 5, 1933. Given Progynon-B December 16, 1933–March 23, 1934, at rate of 90 rat units daily.

Result: Menstruation-like bleeding January 1–3, 1934; again February 5–6 and March 22–26.

Interpretation: first menses during treatment not delayed; second 6 days late (not significant?); third 17 days late, occurring during treatment.

No. 102. For control periods, see protocol of same animal below, p. 248.

Last control menses began November 20, 1933. Given Progynon-B December 18–January 10, 1934, at rate of 30 rat units per day; January 10–April 4, 60 rat units per day.

Result: Menstruation-like bleeding January 10–13, February 5–7, February 20–24, April 5–13.

Interpretation: during treatment there were four cycles of 26, 25, 15 and 44 days respectively, i.e., there was no significant disturbance of rhythm.

No. 105. Control periods:

Season 1932–33. Intervals of 30, 32, 28, 75 days.

Season 1933–34. 31, 29, 29, followed by experiment.

Last control menses began November 22, 1933. Given Progynon-B December 16–January 24, 1934, at rate of 30 rat units daily; January 25–April 13, 60 rat units daily.

Result: Menstruation-like bleeding December 22–23, January 26–27. No further bleeding during treatment; menstruation-like bleeding April 23–26.

Interpretation: first two menses due after beginning of treatment occurred approximately on time; amenorrhea thereafter; but since this animal had a 75-day interval at the corresponding time in the control season 1932–33, the amenorrhea during the experiment cannot be considered significant.

No. 106. For control periods, see protocol of same animal below, p. 249.

Last control menses began December 29, 1932. Given Progynon benzoate January 10–February 23, 1933, at rate of 30 rat units per day.

Result: no bleeding occurred during the treatment. Menstruation-like bleeding February 27 to March 9, beginning 4 days after cessation of treatment.

Interpretation: possibly inhibition of menstruation, including the first expected period.

PART II. Experiments with progestin.

No. 66. Control periods:

Season 1930–31. Intervals of 45, 35, 32, 21, 84, 48 days.

Season 1931–32. 28, 49, 39, 20, 23, 30, 24.

Season 1932–33. 32, followed by experiment with Progynon (p. 245).

Season 1933–34. 19, 29, 67, 28, 13, 37.

Season 1934–35. 30, 24, 23, 29.

Cycle length too irregular to enable prediction of next menses.

Last control menses began February 8, 1935. Given progestin 1 Rab. U. beginning March 2. No menstruation occurred, and the last injection was given March 19. A few erythrocytes appeared in the vaginal washings on March 23, and definite external bleeding resembling menstruation occurred from March 26 (7 days after the last injection of progestin) to March 29.

Biopsy of uterus on morning of March 26; the histological picture was that of early

menstruation from an endometrium which showed evidences of the premenstrual state. Bleeding continued until March 30. Typical menses April 25-27 (interval 30 days).

No. 70. Control periods:

- Season 1931-32. (November date lost). Intervals of 29, 36, 36, 37, 35 days.
- Season 1932-33. 30, 29 days, followed by experiment with Progynon (p. 246).
- Season 1933-34. 28, 28, 29, 29, 29, followed by experiment with Progynon (p. 246).
- Season 1934-35. 32 days.

Usual cycle length (past two years) 28-32 days.

Last control period began November 23, 1934; next period due to begin therefore between December 21 and December 25. Given progestin 1 Rab. U. daily beginning December 20. Microscopic bleeding December 27; a few erythrocytes in vaginal washings December 28-29. Definite menstruation did not occur. Last injection January 9, 1935. Typical menstruation January 16-24 (after 54-day interval). Next menstruation February 16-25 (31-day interval). The animal was then given a second course of progestin.

No. 70, second experiment. Control periods: as above, with the addition of the 31-day interval of January-February, 1935.

Last control menses began February 16, 1935; next menses due to begin between March 16 and March 20. Given progestin 0.5 Rab. U. daily beginning March 7. A few erythrocytes appeared in the vaginal washings beginning March 21, but no visible tinge of blood was seen. Last injection March 31. Externally visible bleeding occurred April 2-10, but this part of the experiment was complicated by the introduction of experimental procedures not relevant to the present discussion.

No. 90. Control periods:

- Season 1932-33. Intervals of 25, 28 days, followed by experiment with Progynon (p. 246).
- Season 1933-34. 31, 28, 26, 27, 27 days, followed by experiment with Progynon (p. 247).
- Season 1934-35. 31, 29 days.

Usual cycle length 25-31 days.

Last control menses began November 23, 1934; next menses due to begin therefore between December 18 and December 24 (had not begun December 20). Given progestin 0.5 Rab. U. daily beginning December 20. Typical menstruation December 26-31 (after interval of 33 days). Last injection January 9. Typical menstruation January 14-16 (interval of 19 days), February 5-9 (interval 20 days), April 5-10 (interval 59 days).

No. 102. Control periods:

- Season 1932-33. Intervals of 28, 28, 15, 41, 19, 30 days.
- Season 1933-34. 32, 29, 27, followed by experiment with Progynon (p. 247).
- Season 1934-35. 33, 31, 27 days.

Usual cycle length 27-33 days.

Last control menses began January 2, 1935; next menses due to begin therefore between January 27 and February 4. Given progestin 1 Rab. U. daily beginning January 21. Menstruation did not occur during treatment. Last injection February 11. Typical menstruation February 16-21 (after 45-day interval). Next menstruation March 16-23 (interval 28 days), April 12-18 (interval 27 days).

No. 106. Control periods:

Season 1932-33. Intervals of 23, 28 days, followed by experiment with Progynon (p. 247).

Season 1933-34. 22, 29, 29, 32, 29, 30, 25.

Season 1934-35. 29, 32.

Usual cycle length 22-32 days; modal length about 30 days.

Last control menses began November 26, 1935; next menses due to begin therefore between December 18 and December 28, probably about December 26. Given progestin 0.1 Rab. U. daily beginning December 20. Typical menstruation December 26-29 (after interval of 30 days). Next menstruation January 28-30, 1935 (interval 33 days). The animal was then given a second course of progestin.

No. 106, second experiment. Control periods: as above, with the addition of the 30- and 33-day intervals of December 1934-January 1935.

Last control menses began January 28, 1935; next menses due to begin therefore between February 19 and March 1, probably about February 27. Given progestin 1 Rab. U. daily beginning February 20. No bleeding having occurred by March 2, the animal was explored under ether. At this time the menses were overdue. A large corpus luteum was found in the left ovary, which was removed for study. On section this corpus luteum proved to be in early degeneration corresponding to corpora of about the second day of natural menstruation, in the author's collection. A sample of the endometrium was removed from the uterus at this exploration, and was found to be in a typical advanced premenstrual state, showing as yet no evidence of menstrual hemorrhage or breakdown.

Externally visible bleeding occurred from March 3 to March 11, and microscopic bleeding thereafter until March 22. How much of this was due to the biopsy incision in the uterus could not of course be determined. Last injection of progestin March 20; macroscopic bleeding March 23-27. Typical menses April 12-19.

No. 115. Control periods:

Season 1933-34. Intervals of 28, 52, 27, 26, 24, 32 days.

Season 1934-35. 30, 25, 25.

Usual cycle length, 24-32 days.

Last control menses began January 7, 1935; next menses due to begin therefore between January 30 and February 8. Given progestin 1 Rab. U. daily beginning January 28. Menstruation did not occur during treatment. Last injection February 18. Typical menstruation February 26-March 2 (after 50-day interval). Next menses March 27-30 (interval 29 days), April 22-25 (interval 26 days).

No. 116. Control periods:

Season 1932-33 (first menstruation in March). Intervals of 28, 29, 29 days.

Season 1933-34. 25, 25, 27, 25, 23, 26, 24 days.

Season 1934-35. 35, 25, 25 days.

Usual cycle length, 23-27 days.

Last control menses began January 7, 1935; next menses due to begin therefore between January 30 and February 3. Given progestin 1 Rab. U. daily beginning January 28. Slight microscopic bleeding was noted in the vaginal smears almost daily from February 7 to February 16, but definite menstruation did not occur. Last injection February 18. Typical menstruation February 23-March 2 (after 47-day interval). Next menses March 25-29 (interval 30 days), April 22-25 (interval 26 days).

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